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Journal canadien des sciences halieutiques et aquatiques

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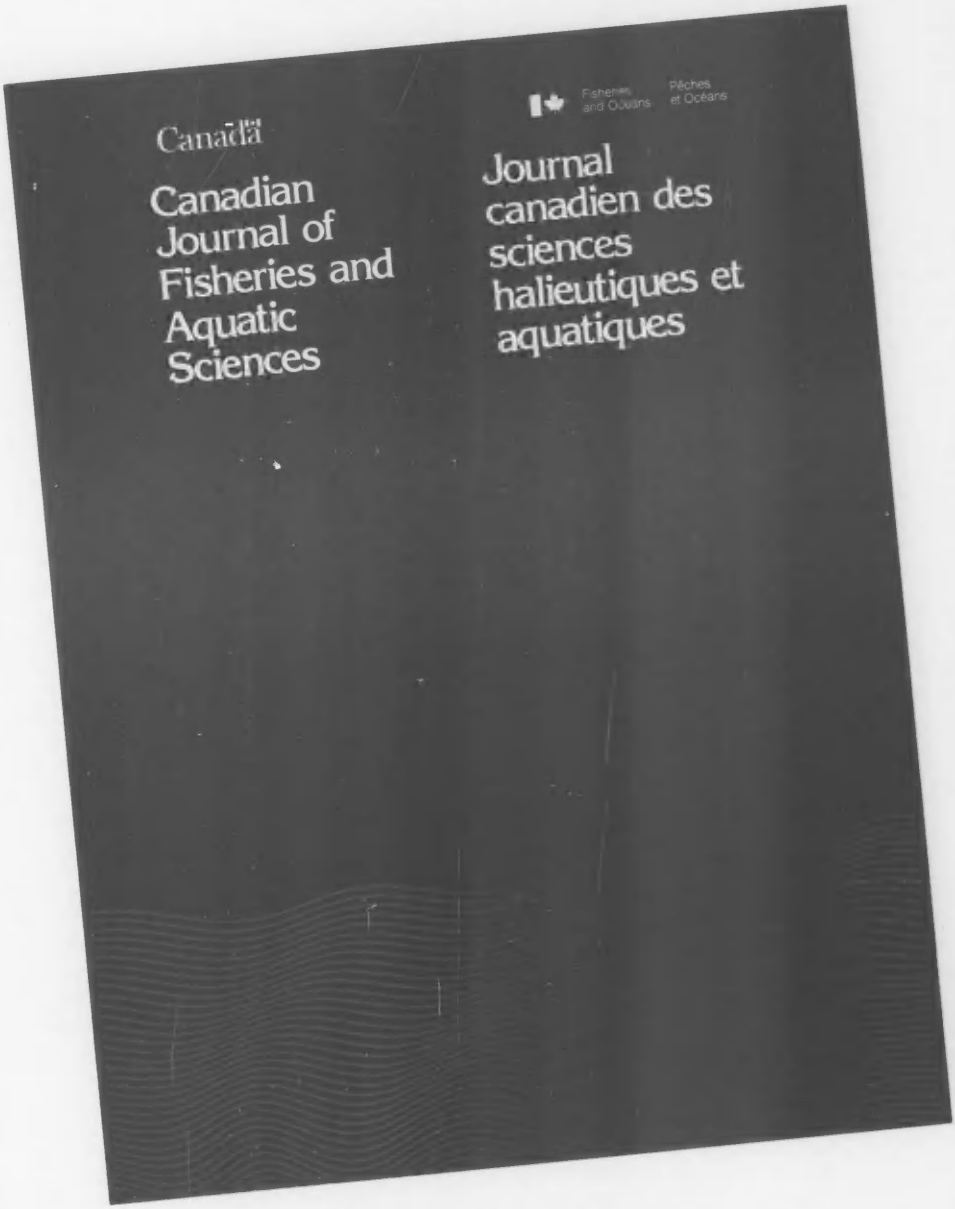
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Orthophosphate turnover time in shallow productive lakes	E.E. Prepas
Coastal water mass replacement: its effects on zooplankton dynamics and the predator-prey complex associated with larval capelin *(Mallotus villosus)*	K.T. Frank and W.C. Leggett
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Canadian Journal of Fisheries and Aquatic Sciences

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Journal canadien des sciences halieutiques et aquatiques

Volume 40, n° 12, décembre 1983

Announcements/Avis

Index

An author–subject index and list of all publications issued during 1983 will be supplied in early 1984, and distributed free to all *Journal* recipients, including subscribers. Additional copies will be available through the Scientific Information and Publications Branch.

Index

Un index par sujet et auteur et une liste de toutes les publications parues en 1983 paraîtront au début de 1984 et seront distribués gratuitement à tous ceux qui reçoivent le *Journal*, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires à la Direction de l'information et des publications scientifiques.

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Editorial

Several years ago the *Journal* adopted the Système International d'Unités (SI) with the *Canadian Metric Practice Guide* (Canadian Standards Association 1979) as the standard. Wholesale adoption of the standard has been strongly resisted by some scientists, however, as some SI units introduce error and may fail to reflect the biological and physical realities the units purport to measure. After thoroughly discussing this problem with the Editorial Board at its 1983 meeting, we decided to retain a few non-SI units.

Calorie — Continued use of the metric unit, calorie, rather than the SI unit, joule, recognizes that conversion between mechanical energy and heat does not function with equal efficiency in both directions; although mechanical energy can be converted to heat with virtually complete efficiency under certain experimental conditions, the converse is not true because some heat is always dissipated. No universally applicable conversion factor exists for the change from heat to mechanical work, hence the belief by some researchers that the calorie is superior to the joule for bioenergetics studies. This issue is probed in depth by Moore (1981). Authors are welcome to use either calories or joules. Calorie should be spelled with a lower case "c" unless reference to kilocalories is intended.

Einstein (Ei) — Photosynthetically active radiation (PAR) can be expressed as $W \cdot m^{-2}$ (or $J \cdot m^{-2} \cdot s^{-1}$) or Ei $(area)^{-1} \cdot (time)^{-1}$, depending on whether energy of incident photons or number of incident photons is to be emphasized. Only the Einstein, a dimensionless number equal to the number of incident protons divided by 6.024×10^{23} (the Avogadro number), expresses photon flux and is not readily converted to $W \cdot m^{-2}$ unless the light measured is monochromatic. The *Journal* will allow use of any of the aforementioned units, according to the author's preference.

Equivalent — The Canadian Metric Practice Guide recommends the mole per cubic metre ($mol \cdot m^{-3}$) as the unit of concentration to supercede the equivalent weight (the weight in grams of an element that combines with or replaces 1 g of hydrogen). However, moles do not always accurately reflect the behavior of biologically important molecules which may differ depending on the pH and chemical environment. For example, the net charge on a protein enzyme molecule will vary with pH, and so will the dynamics of reactions catalyzed by that enzyme. In the case of alkalinity, use of millimoles is ambiguous and can denote misleading quantities of base present in some types of lakes, whereas milliequivalents is an unambiguous quantity and cannot be altered by the conversion of carbonate to bicarbonate. Moreover, if specialized methods are used which by definition deal with charged equivalents, then use of milliequivalents is mandatory. If recourse to milliequivalents is not necessary, authors will be expected to use millimoles.

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Éditorial

Il y a plusieurs années, le *Journal* adoptait le Système International d'Unités (SI), selon les normes contenues dans le Guide de familiarisation au système métrique du Canada (Association canadienne de normalisation 1979). Certains scientifiques ont cependant résisté à l'adoption d'emblée de ces normes, car quelques-unes de ces unités sont la cause d'erreurs et peuvent ne pas refléter les réalités biologiques et physiques qu'elles sont supposées mesurer. Après avoir soigneusement examiné ce problème avec le Conseil de rédaction à sa réunion de 1983, nous avons décidé de retenir quelques unités non SI.

Calorie — Le maintien de l'unité métrique, la calorie, plutôt que l'unité SI, le joule, tient compte du fait que la conversion entre l'énergie mécanique et la chaleur ne se fait pas avec la même efficacité dans les deux sens. Bien que l'énergie mécanique se convertisse en chaleur avec une efficacité à peu près totale, l'inverse n'est pas vrai car une certaine quantité de chaleur est toujours dissipée. Il n'existe pas de facteur de conversion universellement applicable pour le passage de la chaleur à un travail mécanique, et c'est pourquoi certains chercheurs croient que la calorie est supérieure au joule dans des études bioénergétiques. Moore (1981) traite de cette question en profondeur. Les auteurs peuvent donc utiliser soit la calorie ou la joule. Calorie devrait s'écrire avec un «c» minuscule, à moins qu'il ne s'agisse de kilocalories.

Einstein (Ei) — La radiation photosynthétiquement active (PAR) peut s'exprimer par $W \cdot m^{-2}$ (ou $J \cdot m^{-2} \cdot s^{-1}$) ou par Ei $(aire)^{-1} \cdot (temps)^{-1}$, selon qu'on veuille mettre l'accent sur l'énergie des photons incidents ou sur le nombre de ces derniers. Seul le Einstein, un nombre dépourvu de dimensions égal au nombre de protons incidents divisé par $6{,}024 \times 10^{23}$ (nombre d'Avogadro) exprime le flux photonique, et ne peut être facilement converti en $W \cdot m^{-2}$, à moins que la lumière mesurée ne soit monochromatique. Le *Journal* permettra l'emploi de l'une ou l'autre des unités mentionnées ci-dessus, selon la préférence de l'auteur.

Équivalent — Le guide de familiarisation au système métrique recommande la mole par mètre cube ($mol \cdot m^{-3}$) comme l'unité de concentration pour remplacer le poids équivalent (le poids en grammes d'un élément qui se combine avec, ou qui remplace 1 g d'hydrogène). Cependant, les moles ne reflètent pas toujours avec précision le comportement de molécules biologiquement importantes qui peuvent différer selon le pH et le milieu chimique. Par exemple, la charge nette sur une molécule d'enzyme protéique variera en fonction du pH, de même que la dynamique des réactions catalysées par cette enzyme. Dans le cas de l'alcalinité, l'emploi de millimoles est ambigu et peut dénoter des quantités trompeuses de base présente dans certains types de lacs, alors que milliéquivalents est une quantité ambiguë et ne peut être modifiée par conversion de carbonate en bicarbonate. En outre, si l'on utilise des méthodes spécialisées qui font appel à des équivalents chargés, l'emploi de milliéquivalents est alors obligatoire. Quand il n'est pas nécessaire d'avoir recours aux milliéquivalents, les auteurs devraient employer millimoles.

New Practical Salinity Scale — In 1962, efforts to formulate internationally accepted standards were undertaken by a committee, later to become known as the Joint Panel on Oceanographic Tables and Standards (JPOTS), jointly appointed by UNESCO, ICES (International Council for the Exploration of the Sea), SCOR (Scientific Committee on Oceanic Research), and IAPSO (International Association for the Physical Sciences of the Ocean). The establishment of JPOTS was predicated on the need for a more precise definition of the fundamental quantities of seawater — salinity, conductivity, and density — and establishment of more precise relationships between them in terms of temperature and pressure. To establish these relationships, JPOTS promoted work in specified laboratories which led to formulation of the New Practical Salinity Scale and standardization of other measurements. Rather than being based on the original notion of the ratio of mass of salt in a given seawater sample to the total mass of the sample, the practical salinity (S) of a sample is now based on measurements defined in terms of the ratio (K_{15}) of the electrical conductivity of the seawater sample at 15°C and 1 standard atmosphere of pressure to that of a KCl solution in which the mass fraction of KCl is 32.4356×10^{-3} at the same temperature and pressure. The K_{15} value exactly equal to 1 corresponds, by definition, to a practical salinity exactly equal to 35. Salinities on the new scale are now dimensionless numbers (e.g. $S = 35$) and are no longer expressed as ‰. The background and rationale for this change is complex and is presented in Lewis (1982) and the IMS Newsletter (1982). The new salinity scale has been adopted by most oceanographers, and all major marine data centers are requiring that salinity data be given in the new units to facilitate data exchange. The *Journal* thus recommends that the New Practical Salinity Scale be adopted and phased in beginning in January 1984. Authors should mention how their salinities were calculated and provide conversions from parts per thousand to the new scale, if possible.

Volume flux — Volume flux in oceanography has been expressed in millions of cubic metres per second ($10^6\ m^3 \cdot s^{-1}$) but later, for ease of use, the Sverdrup (Sv) was introduced ($Sv = 10^6\ m^3 \cdot s^{-1}$). However, this unit is not internationally recognized by scientists other than oceanographers and has no official standing. JPOTS recommended that the Sverdrup be dropped in favor of $10^6\ m^3 \cdot s^{-1}$, and the editors endorse that recommendation.

Geopotential — Geopotential difference is often referred to as dynamic height or dynamic depth ($= 10\ m^2 \cdot s^{-2}$), but use of this term is discouraged by JPOTS, since the quantity described has the dimensions and meaning of an acceleration potential (i.e. a pressure) and not those of height. It should be dropped and replaced by $J \cdot kg^{-1}$.

As always, readers' reactions to this editorial are welcomed, as are assessment and constructive criticisms of the *Journal*'s policies.

CANADIAN STANDARDS ASSOCIATION. 1979. Canadian metric practice guide. National Standard of Canada CAN3-Z234.1-79: 67 p. (Available from Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3)

Nouvelle échelle de salinité pratique — On formait en 1962 un comité qui eut pour mandat de formuler des normes internationalement acceptables. Ce comité prit par la suite le nom de Comité conjoint des tables et normes océanographiques (JPOTS), nommé conjointement par l'UNESCO, le CIEM (Conseil international pour l'exploration de la mer), SCOR (Comité scientifique pour les recherches océaniques) et IAPSO (Association internationale des sciences physiques de l'océan). Le JPOTS a été établi à cause du besoin d'une définition plus précise des quantités fondamentales de l'eau de mer — salinité, conductivité et densité — et l'établissement de relations plus précises entre ces quantités en termes de température et de pression. Pour établir ces relations, JPOTS a encouragé des travaux dans des laboratoires particuliers, travaux qui ont abouti à la formulation d'une nouvelle échelle de salinité pratique et la standardisation d'autres mesures. Au lieu de reposer sur la notion originelle de rapport de la masse de sel dans un échantillon d'eau de mer donné à la masse totale de l'échantillon, la salinité pratique (S) d'un échantillon est maintenant fondée sur des mesures définies en termes du rapport (K_{15}) de la conductivité électrique de l'échantillon d'eau de mer à 15°C et une atmosphère standard de pression à celle d'une solution de KCl dans laquelle la fraction de masse de KCl est $32{,}4356 \times 10^{-3}$ aux mêmes température et pression. Une valeur de K_{15} exactement égale à 1 correspond, par définition, à une salinité pratique équivalent exactement à 35. Sur la nouvelle échelle, les salinités sont maintenant des nombres dépourvus de dimensions (p. ex., $S = 35$) et ne sont plus exprimées comme ‰. La documentation et les raisons de ce changement sont complexes, et on les trouvera dans Lewis (1982) et dans le IMS Newsletter (1982). La plupart des océanographes ont adopté la nouvelle échelle de salinité et, afin de faciliter les échanges de données, les principaux centres de données océanographiques exigent que la salinité soit donnée dans ces nouvelles unités. Le *Journal* recommande donc que la nouvelle échelle de salinité pratique soit adoptée et introduite graduellement à partir de janvier 1984. Les auteurs devraient mentionner la façon dont ils ont calculé leurs salinités et fournir, si possible, des facteurs de conversion, de partie par mille à la nouvelle échelle.

Flux volumique — Le flux volumique en océanographie a été exprimé en million de mètres cubes par seconde ($10^6\ m^3 \cdot s^{-1}$), mais plus tard, pour simplifier, on a introduit le Sverdrup (Sv) ($Sv = 10^6\ m^3 \cdot s^{-1}$). Cette unité n'est toutefois pas reconnue internationalement par les scientifiques autres que les océanographes et n'a pas de statut officiel. Le JPOTS recommande de laisser tomber le Sverdrup en faveur de $10^6\ m^3 \cdot s^{-1}$, et les rédacteurs endossent cette recommandation.

Géopotentiel — On réfère souvent à la différence de géopotentiel comme étant la hauteur dynamique ou la profondeur dynamique ($= 10\ m^2 \cdot s^{-2}$), mais le JPOTS est d'avis que ce terme devrait être abandonné car la quantité a les dimensions et la signification d'un potentiel d'accélération (par ex. une pression) et non celles de la profondeur. Il devrait être remplacé par $J \cdot kg^{-1}$.

Comme toujours, tout commentaire de la part des lecteurs

IMS NEWSLETTER. 1982. Vol. 30. UNESCO, Paris. (Available from UNESCO, 7 Place de Fontenoy, 757 Paris, France)

LEWIS, E. L. 1982. The practical salinity scale of 1978 and its antecedents. Mar. Geod. 5(4): 351–358.

MOORE, T. 1981. Units of energy — what's in a name? New Sci. 89(1239): 344–346.

JOHANNA M. REINHART

Editor

sur cet éditorial sera le bienvenu, comme d'ailleurs l'évaluation et les critiques constructives des politiques du *Journal*.

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Optimal Fisheries Investment: Comparative Dynamics for a Deterministic Seasonal Fishery

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A dynamic fisheries model is developed to simultaneously optimize investment in the resource stock (the fish) and investment in the capital stock (the fleet). Each of these investment problems faces a major complication; investment in the resource is constrained by the natural population dynamics, while investment in the physical capital stock tends to be irreversible because capital used in natural resource industries is often nonmalleable. The model assumes a seasonal fishery in which annual escapement and capital investment levels can be controlled. A dynamic programming approach is used to analyze the model heuristically and numerically. The comparative dynamics of optimal investment strategies are studied, with regard to (i) delays in investment, (ii) population dynamics parameters, (iii) fish price, (iv) capital cost, (v) depreciation rate, and (vi) discount rate. In particular, the depreciation rate and the ratio of unit capital costs to unit operating costs play interesting and complex roles in determining optimal investment levels.

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Un modèle de pêche dynamique a été élaboré dans le but d'optimiser l'investissement dans la ressource (le poisson) en même temps que l'investissement en capital (la flottille). Chacun de ces investissements rencontre une complication majeure : l'investissement dans la ressource doit subir la contrainte de la dynamique de population naturelle, alors que l'investissement en capital physique tend à être irréversible parce que, souvent, le capital utilisé dans les industries de ressources naturelles est non malléable. Le modèle suppose une pêche saisonnière dans laquelle peuvent être réglés les niveaux d'échappement annuel et de mise de fonds. On utilise une programmation dynamique pour analyser le modèle de manière heuristique et numérique. La dynamique comparative des stratégies d'investissement optimales est étudiée sous les aspects suivants : (i) retards dans l'investissement, (ii) paramètres de dynamique de population, (iii) prix du poisson, (iv) coût en capital, (v) taux d'amortissement et (vi) taux d'escompte. Le taux d'amortissement, en particulier, ainsi que le rapport du coût en capital unitaire au coût d'opération unitaire jouent des rôles intéressants et complexes dans la détermination des niveaux d'investissement optima.

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THE chronic problem of overcapacity in fisheries has been well documented, but analytical studies of optimal fleet sizes and fisheries investment strategies have been rare. On the other hand, determination of optimal investment levels and optimal capital stocks is a popular topic in the economics literature, but with few exceptions little effort has been made to apply this theory to renewable resource management.

The difficulties in utilizing economic theory to deal with fisheries investment problems are twofold. First, fisheries tend to be faced with the problem of nonmalleability of capital; specialized fishing boats, like forestry and mining machinery, often have few if any alternative uses. Hence, investment is irreversible — capital cannot be removed from the fishery except through natural depreciation. This problem of irreversibility was discussed in general terms by Arrow (1968) and in the context of exhaustible resource production by Campbell (1980) and Lasserre (1983). Second, in fisheries management the question of investment in the capital stock (the fleet) cannot be separated from "investment" in the resource stock (the fish), the latter being accomplished by

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controlling harvest rates or escapements. The resulting joint investment problem is faced each season; decisions regarding desired fleet size and desired fish stock size must be made simultaneously.

Due to these complexities, most fisheries management models to date have concentrated on the resource stock, treating the capital stock as given. Those that have considered capital investment explicitly have generally used simplifying assumptions to avoid either the irreversibility problem or the dynamic joint investment problem described above. An example of the former is that of Smith (1968), in which both the fish population and the fleet size vary over time, but capital is treated as malleable (an assumption that could be reasonable for fisheries that have access to the capital stock of a larger "neighboring" fishery). The latter problem of dynamic joint investment is avoided if investment can be assumed to be a once-and-for-all irreversible decision at the outset (Clark and Kirkwood 1979; Dudley and Waugh 1980; Silvert 1977); this would be the case, for example, if future fish populations are independent of current stock levels. Naturally, neither of the above simplifications applies in general. A more complete analysis has been undertaken by Clark et al. (1979), who solved a continuous-time deterministic version of the irreversible investment problem, with two state variables (biomass and fleet capacity) and two decision variables (fishing effort and investment) varying over time. Recently, McKelvey (1983) studied a similar model, involving the optimal mix of "specialist" and "generalist" vessels in a fishing fleet.

This paper extends the study of optimal capital investment in renewable resource industries by expanding the work of Clark et al. (1979). The model here is similar to theirs but more realistic in a number of respects: (i) a year-by-year time frame is used, with fishing taking place continuously within each season, (ii) the decision variables are end-of-season escapement and yearly investment (as opposed to instantaneous fishing effort and investment in the CCM continuous-time case), and (iii) delays are allowed between the time at which investment decisions are made and the time at which these investments come online.

In addition, a dynamic programming approach is used to study arbitrary stock-recruitment functions, including the Beverton–Holt and Ricker forms, and to obtain detailed comparative dynamics results. Specifically, this paper describes the effects on optimal investment/escapement policies of the following factors: (i) discrete-time versus continuous-time analysis, (ii) investment delays, (iii) productivity and carrying capacity of the resource stock, (iv) fish price, (v) capital cost, (vi) discount rate, and (vii) depreciation rate. In a companion paper (Charles 1983), a stochastic version of the model is used to study the role of uncertainty in fisheries investment problems.

The Model

An aggregated (single-species single-cohort) fish stock is assumed, with the biomass at the beginning of season $n + 1$, R_{n+1}, depending on the end-of-season escapement from the previous season, S_n, according to the stock-recruitment relationship $R_{n+1} = F(S_n)$. In applications discussed here, this reproduction function will be either pure compensatory ($F' > 0, F'' < 0$) or overcompensatory, using the Beverton–Holt or Ricker form, respectively (Beverton and Holt 1957; Ricker 1954). Natural mortality is constrained to occur at the end of the fishing season, but fishing mortality occurs continuously during the season, with biomass following the common differential equation $dx/dt = -h(t) = -qE(t)x(t)$, where $h(t)$ is the harvest rate, $E(t)$ is the instantaneous aggregated fishing effort, q is a constant catchability coefficient, and initially $x(0) = R_n$ in year n. The escapement is then $S_n = R_n \exp\left[-q\int_0^T E(t)dt\right]$ where T is a fixed maximum season length.

The capital stock, or fleet capacity, K, is represented here by the maximum instantaneous fishing effort; at any point t in the season n, effort is constrained by $0 \leq E(t) \leq K_n$. Hence, K_n depicts the catching power of the fleet, an aggregated measure including fishing vessels, together with nets, machinery, engines, and training of the fishermen.

For simplicity, the cost per unit of new fleet capacity is assumed to be a constant, δ, irrespective of the current level of capacity. Furthermore, it is assumed that this cost must be paid in full at the time the new capacity is ordered. The unit capital cost may be considered to include a fraction of processing capacity costs, where appropriate. (In practice, the type of vessel, or the mix of vessel types, chosen for a fishery may affect the unit operating cost and the unit capital cost in different ways. This complication is not included here; the fleet is taken to be homogeneous.)

Depreciation is assumed to occur at the end of each season, with a constant fraction γ (the depreciation rate) of the current capital stock wearing out or otherwise being removed from the fishery at that time.

Perhaps the most important assumption in this model, as in the Clark et al. (1979) model, is the irreversibility of investment. It is assumed here that the fleet capacity cannot be decreased at will but only through the process of depreciation. Hence, the dynamics of the capital stock, K, can be expressed as follows:

$$K_{n+1} = (1 - \gamma)K_n + I_{n+1}; \quad I_{n+1} \geq 0$$

where the investment I_{n+1} becomes available in year $n + 1$. (This key irreversibility assumption could be relaxed somewhat given either a positive scrap value for fishing capital (see Clark et al. 1979, p. 35–37) or the possibility of bringing outside vessels, either domestic or foreign, into the fishery on a temporary basis (see, e.g., McKelvey 1981). In any case, investment is not entirely irreversible in the model, since capital depreciates annually, as is the situation in real-world fisheries.)

A further consideration in dealing with investment policies is the possibility of a delay existing between the time an investment decision is made and the time the corresponding new capacity becomes available. Such delays may arise due to the time necessary to construct new vessels and/or transport them to the fishing grounds. In a deterministic world, such investment delays increase the effective capital cost and change the appearance of optimal policies but do not affect the substance of the management problem. In this paper the cases

of instantaneous and delayed investment are compared, but primary emphasis is placed on the more realistic delayed investment assumption. To simplify the structure of the model while incorporating a reasonable delay, it is assumed that in any given year the decision regarding next season's optimal capacity must be made before the end of the current season and that full payment for any new investment must be made in the current season.

The fishery management problem involves yearly escapement and investment decisions. The timing of the two decisions depends on the assumption regarding delays in bringing investment online; the following applies to the delayed investment case. Given the recruitment R_n, the optimal escapement S_n^* is chosen, subject to the constraint $R_n e^{-qTK_n} \leq S_n^* \leq R_n$ where the lower limit is reached by fishing with maximum effort throughout the season. Then, the optimal investment for next year is chosen, subject to $I_{n+1} \geq 0$ and based on the value of S_n^*; payment is made in year n for this new capacity.

The fishery is assumed to face perfectly elastic demand (with given constant selling price p) and linear operating costs (with unit cost of effort c). Yearly rents accruing to the fleet, as a function of recruitment, capacity, escapement, and investment, are then given by

$$\pi(R,K,S,I) = \int_0^T (pqEx - cE)dt - \delta I$$
$$= p(R - S) - (c/q) \log (R/S) - \delta I$$

using $dx/dt = -qEx$, $x(0) = R$, $x(T) = S$. Assuming that the fishery manager desires to maximize the discounted sum of annual fishery rents, our problem can be stated as follows:

$$\underset{(S_1,I_2,S_2,\ldots)}{\text{Max}} \left[\sum_{n=1}^{\infty} \alpha^{n-1}\, \pi(R_n,K_n,S_n,I_{n+1})\right]$$

subject to $R_{n+1} = F(S_n)$, $K_{n+1} = (1 - \gamma)K_n + I_{n+1}$, $R_n e^{-qTK_n} \leq S_n \leq R_n$, $I_{n+1} \geq 0$ where α is the annual discount factor.

The dynamic programming equation for the value of the fishery in state (R_n,K_n) at the start of a season n is given by

$$V(R_n,K_n) = \underset{S_n}{\text{Max}}\ \underset{I_{n+1}}{\text{Max}}\ [\pi(R_n,K_n,S_n,I_{n+1}) + \alpha V(R_{n+1}, K_{n+1})]$$

where $R_{n+1} = F(S_n)$, $K_{n+1} = (1 - \gamma)K_n + I_{n+1}$, the outer maximization is subject to $R_n e^{-qTK_n} \leq S_n \leq R_n$, and the inner maximization is over the range $I_{n+1} \geq 0$. This is simply a statement, using Bellman's (1957) principle of optimality, that the value of the fishery is given by the maximum value of the sum of current rents plus the discounted future value of the fishery, where the escapement and investment levels are chosen from the set of all feasible values. Removing the subscripts on the variables, this can be rewritten as

$$(1)\quad V(R,K) = \underset{R\cdot\exp(-qTK)\leq S\leq R}{\text{Max}}\ \underset{I\geq 0}{\text{Max}}\ \{\pi(R,K,S,I) + \alpha V[F(S), (1 - \gamma)K + I]\}$$

where $\pi(R,K,S,I) = p(R - S) - (c/q) \log (R/S) - \delta I$.

Equation 1 will form the basis for most of the analysis and results presented in this paper. For convenience, a full list and definition of symbols used in this paper follows:

R	Annual recruitment
K	Fleet capacity (capital stock)
S	Annual escapement
I	Annual investment
x	Instantaneous biomass (in-season)
E	Instantaneous fishing effort
$V(R,K)$	Value function
V_R	Partial derivative of V with respect to R
V_K	Partial derivative of V with respect to K
$s(\cdot)$	Target escapement function
$h(\cdot)$	Target fleet capacity function
$F(\cdot)$	Stock-recruitment function
a	Maximum productivity of the fish stock
b	Maximum possible recruitment
m	Instantaneous natural mortality rate
T	Maximum possible season length
q	Instantaneous catchability coefficient
p	Unit market price for "fish"
c	Unit cost of fishing effort
δ	Unit cost of capital
γ	Annual depreciation rate
α	Annual discount factor = $1/(1 +$ discount rate$)$
π	Annual fishery rents
x_0	Bionomic zero-profit biomass level = c/pq

Heuristic Analysis and Numerical Method

To gain qualitative information about the optimal investment and escapement problem, this section begins with an heuristic study of the dynamic programming equation 1. Assume for now that the fish stock displays pure compensatory population dynamics (i.e. a concave increasing stock-recruitment function, as in the Beverton–Holt model) and that $V_R > 0$, $V_K > 0$, $V_{RK} > 0$, and $V_{KK} < 0$ over all nonzero values of R and K for which V is twice differentiable. The latter assumption simply states that more fish and more capital increase the value of the fishery, that more fish are more desirable the larger the capital stock, and that the fishery has decreasing marginal returns to capital.

Optimal Investment

Performing the inner maximization in equation 1, for fixed S, produces the optimality equation for investment:

$$(2)\quad V_K[F(S), (1 - \gamma)K + I^*] = \delta/\alpha \quad \text{or } I^* = 0 \text{ if } V_K[F(S), (1 - \gamma)K] < \delta/\alpha.$$

This states that, unless the fleet is temporarily overcapitalized, next year's optimal capacity, $(1 - \gamma)K + I^*$, should be set such that the marginal benefit of an extra unit of capital equals its marginal cost.

Define $K' = h(S)$ as the solution of the implicit equation $V_K[F(S), K'] = \delta/\alpha$. Then, $h(S)$ is next season's optimal capacity, which is an increasing function of escapement by the above assumptions (Charles 1982).

Thus, if $(1 - \gamma)K > h(S)$, the optimal investment is $I^* = 0$ (capital is already sufficiently abundant), while otherwise, I^* is chosen so that $(1 - \gamma)K + I^* = h(S)$. This can be written as

$$(3) \quad I^*(S,K) = \text{Max}\,[h(S) - (1 - \gamma)K, 0]$$

so that, in general, investment desired for the next season depends both on capacity and on escapement in the current season.

Optimal Escapement

Inserting $I^*(S,K)$ into equation 1, performing the outer maximization by taking a total derivative with respect to S, and noting that for any S and K either $I^*_S(S,K) = 0$ or $\alpha V_K[F(S), (1 - \gamma)K + I^*(S,K)] - \delta = 0$, an optimality expression is produced; this equates the marginal benefit and marginal cost of an incremental increase in escapement:

$$(4) \quad \alpha F'(S) \cdot V_R[F(S), (1 - \gamma)K + I^*(S,K)] = p\left(1 - \frac{x_0}{S}\right)$$

where $x_0 = c/pq$ is the bionomic zero-profit biomass level, and the constraint $Re^{qTK} \leq S \leq R$ has been neglected temporarily.

Assume that equation 4 implicitly defines a unique function $S = s(K)$, representing the target escapement for a given capacity level, K. Numerical tests indicate that $s(K)$ is indeed a well-behaved single-valued function. In Charles (1982) it is shown that this target escapement increases with the level of the capital stock, approaching the optimal escapement for the more common abundant-capital problem as the fleet capacity becomes large. In other words, for sufficiently large K, $s(K)$ satisfies the "Modified Golden Rule" equation:

$$F'(S) \cdot \frac{1 - x_0/F(S)}{1 - x_0/S} = 1/\alpha.$$

From a particular state (R,K) of the fishery, the feasible escapement S is constrained by $Re^{-qTK} \leq S \leq R$. Hence, the target $s(K)$ may not always be attainable and the optimal escapement $S^* = S^*(R,K)$ must be defined as follows:

$$(5) \quad S^*(R,K) = \begin{cases} R \cdot \exp(-qTK); & R > s(K) \exp(qTK) \\ s(K); & R \text{ intermediate} \\ R; & R < s(K). \end{cases}$$

This completes, at least heuristically, the overall synthesis of the optimal harvesting/investment policy in the form of the two policy functions $s(K)$ and $h(S)$, giving the optimal action (S^*,I^*) as a function of the state (R,K). In general terms, the optimal escapement is expected to increase with the current capital stock size, while the optimal fleet capacity desired for next season should increase with the current end-of-season escapement. Given the optimal policies $S^*(R,K)$ and $I^*(S,K)$, the resulting value function is defined implicitly by the equation

$$(1') \quad V(R,K) = \pi(R,K,S^*(R,K),I^*[S^*(R,K),K]) + \alpha V(F[S^*(R,K)], (1 - \gamma)K + I^*[S^*(R,K),K])$$

which is rather complex in general but simplifies somewhat in special cases (see last subsection under Numerical Results).

To summarize the behavior of the fishery, at the beginning of a season, given recruitment R and capacity K, the fish stock is first harvested down to an escapement $S^*(R,K)$. Then, depreciation and investment occur such that if the depreciated fleet capacity $(1 - \gamma)K$ is less than the target capital stock $h(S^*)$, investment brings the capacity to $h(S^*)$ by the start of the next season. The process is then repeated from the new biomass/capacity point $\{F[S^*(R,K)], (1 - \gamma)K + I^*[S^*(R,K),K]\}$. The resulting trajectories, and their eventual convergence on a long run equilibrium point, are discussed under Numerical Results.

The above heuristic discussion applies to the delayed investment case. If, instead, investment is assumed to occur instantaneously, appropriate modifications must be made to the analysis (see Charles 1982 for details). It is shown there that the only substantial changes are (i) the optimal fleet capacity target becomes a function of the current season's recruitment rather than the past seasons's escapement, $K^* = h(R)$, and (ii) the effective unit capital cost is reduced from δ to $\alpha\delta$, since new investment is now available immediately.

Numerical Method

The heuristic analysis provides the basis for the numerical methods used to solve the dynamic programming problem, equation 1. In particular, optimal management can be summarized in the form of two policy curves, $s(K)$ and $h(S)$ (or $h(R)$), representing the optimal escapement and capacity targets, respectively. The numerical scheme uses a "policy iteration" methodology to derive these optimal control functions. This approach, discussed extensively in Charles (1982), is simply outlined here. First, an initial guess is made for $s(K)$ and $h(S)$, and the value function V corresponding to these policies is calculated. The partial derivatives V_R and V_K are then deduced and inserted into equations 2 and 4; these are used to obtain improved policies $s(K)$ and $h(S)$. The corresponding new value function is determined and the policy improvement algorithm is repeated until suitable convergence to the optimal functions $s(K)$ and $h(S)$ is achieved.

The numerical scheme for accomplishing this policy iteration process requires discretization of the state variables (biomass and capacity) and use of a two-dimensional grid in the biomass/capacity plane. For each pair of policy functions, a set of simultaneous equations is solved for the value function at grid points, and a smooth surface is formed between these points to obtain the partial derivatives V_R and V_K. Linear or cubic interpolation is used where necessary to deduce values lying between the grid points.

The numerical scheme worked well in all cases involving delayed investment. A modified methodology was required when investment was instantaneous; for the two base cases considered under Numerical Results, this revised method performed well when the fish stock was slow-growing but was ill-behaved in the one fast-growing case. Hence, in this latter

TABLE 1. Base case parameter values for the prawn and whale fisheries. The data are adapted from Clark and Kirkwood (1979) and Clark and Lamberson (1982) (cd, catcher day; BWU, blue whale unit; A$, Australian dollars).

Quantity	Prawn fishery[a]	Whale fishery
"Fish" price (p)	0.9 A$·kg^{-1}	7000 US$·BWU^{-1}
Variable cost (c)	1600 A$·wk^{-1}·vessel^{-1}	5000 US$·cd^{-1}
Capital cost (δ)	0.47×10^{6} A$·vessel^{-1}	10000 US$·cd^{-1}·yr^{-1}
Depreciation rate (γ)	0.15	0.15
Discount factor (α)	0.9	0.9
Catchability (q)	0.00179·wk^{-1}·vessel^{-1}	1.3×10^{-5}·cd^{-1}
Max. season length (T)	26.0 wk	1.0 yr
Nat. mortality rate (m)	0.05·wk^{-1}	0.1·yr^{-1}
Max. productivity (a)	42.0	1.15
Max. recruitment (b)	7.0×10^{6} kg	1.186×10^{7} BWU

case, results reported here are approximate (but do in fact reflect the qualitative behavior deduced in this section).

Numerical Results

A full analysis of the deterministic investment and escapement model embodied in equation 1 can now be provided. Numerical results are based on two fisheries: (i) the Australian Gulf of Carpenteria banana prawn fishery (Clark and Kirkwood 1979) and (ii) the aggregated pelagic whaling fishery (Clark and Lamberson 1982).

The available data for these fisheries have been somewhat simplified to fit the present model and to emphasize the optimal investment problem. In the prawn fishery, a homogeneous fleet is assumed, an average prawn weight used in lieu of intraseasonal growth, and natural mortality is constrained to occur during the off-season. The whaling data used by Clark and Lamberson (1982) have been converted from continuous- to discrete-time and, as in the continuous-time case, delays in recruitment are neglected (cf. Clark 1976).

The stock-recruitment function $F(S)$ is given by $R = F(S) = aS/(1 + aS/b)$ or $R = F(S) = aS\cdot e^{-aS/eb}$ for the Beverton–Holt and Ricker cases, respectively, where S is the escapement after fishing has taken place. The maximum possible recruitment, b, for the prawn fishery is set equal to the sample mean of recruitment data from G. P. Kirkwood (C.S.I.R.O. Division of Fisheries, Cronulla, Australia, personal communication). The maximum rate of growth, or productivity, of the prawn stock, a, is set arbitrarily at the moderately high base value of $a = 42$.

The data used for each fishery are presented in Table 1. If S is the escapement after fishing, Se^{-mT} is taken to be the end-of-year escapement after both fishing and natural mortality. An examination of the stock-recruitment functions indicates that the factor e^{-mT} can be directly incorporated by changing the value of a given in Table 1 to ae^{-mT}; hereafter, the stated value of a is always first transformed to ae^{-mT} before being inserted in the stock-recruitment function $F(\cdot)$. (Note that although the results presented below are based on two fisheries and two stock-recruitment functions, they are in fact quite general. With the choice of the Beverton–Holt or Ricker function, one can capture the qualitative features of most fisheries, and by varying the parameters in the two base case fisheries, arbitrary economic and ecological conditions can be considered.)

It will be of interest to compare optimal investment policies with the open-access scenario resulting from uncontrolled fisheries development. Assuming that in the open-access case investment continues until the average net revenue (per unit capacity) just covers the unit capital cost, then in equilibrium:

$$\frac{\alpha}{1-\alpha}\cdot\frac{\pi(R,K,S,I)}{K} = \delta$$

where the left-hand side represents the total present value of discounted rents, per unit of capital. Setting $I = \gamma K$ to hold the capital stock constant in equilibrium, and assuming full utilization of the fleet, this can be written as

$$\frac{\alpha}{1-\alpha}\cdot[p(R-S)/K - cT - \gamma\delta] = \delta$$

with $R = F(S) = Se^{qTK}$. This simplifies to

$$\frac{(e^{qTK}-1)S}{K} = \left[\left(\frac{1-\alpha}{\alpha}+\gamma\right)\delta + cT\right]\Big/p.$$

Solving this equation simultaneously with the equilibrium condition $F(S)e^{-qTK} = S$ produces the open-access equilibrium capital stock and biomass.

If Beverton–Holt stock recruitment is assumed, so that $F(S) = aS/(1 + aS/b)$, this solution can be simplified. In equilibrium, $F(S)/S = a/[1 + (aS/b)] = e^{qTK}$, so that $S = (b/a)(ae^{-qTK} - 1)$. Hence, the open-access capacity can be restated as the solution of the equation

$$(6)\quad (b/a)(ae^{-qTK}-1)(e^{qTK}-1)/K = \left[\left(\frac{1-\alpha}{\alpha}+\gamma\right)\delta + cT\right]\Big/p.$$

This equation can be solved iteratively for the optimal fleet capacity K and is applied below.

Instantaneous Investment Case

Interpretation of the results is facilitated by comparing with those obtained by Clark et al. (1979), who assumed that

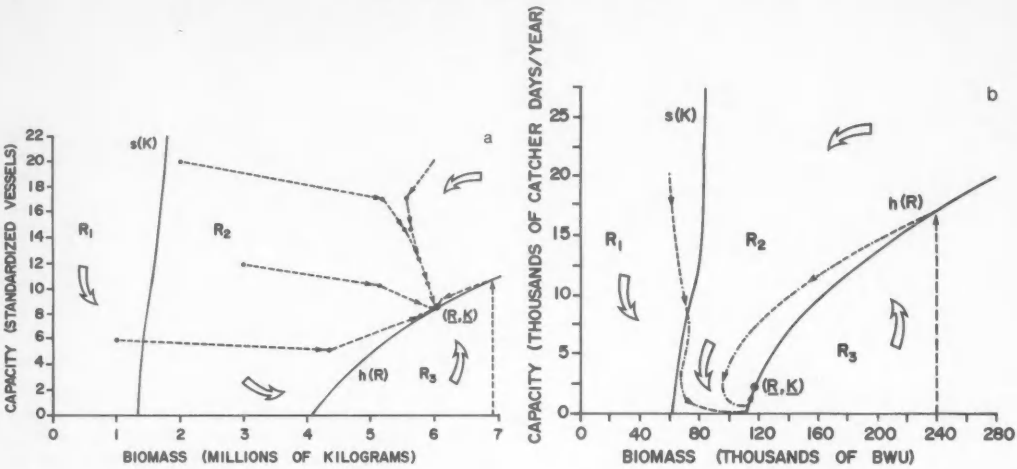

FIG. 1. Optimal capacity function, $h(R)$, and optimal escapement function, $s(K)$, for (a) the base case prawn fishery and (b) the base case whale fishery, with instantaneous investment. Large arrows indicate the overall effect of fishery dynamics. Sample trajectories and the long-run equilibrium $(\underline{R},\underline{K})$ are also shown.

investment occurs instantaneously. Figure 1 here is precisely the discrete-time analogue of the Clark et al. (1979) results (in particular their fig. 2), and the $s(K)$ and h(S) curves correspond closely with their switching curves, σ_1 and σ_2, respectively. As expected, both $s(K)$ and $h(S)$ are increasing functions, $h(S)$ is concave, and $s(K)$ approaches a maximum value at large fleet capacities. To the left of the $s(K)$ curve, in region R_1, neither harvesting nor investment is desirable. In region R_2, harvesting should take place, reducing the fish stock towards the target escapement curve $s(K)$, or as close as possible to that escapement given the level of capacity available. However, as long as the fishery is in region R_2, no new fleet investment should be undertaken. If $K < h(R)$, so that the fishery lies in region R_3, immediate investment should occur until $K = h(R)$, and thereafter, harvesting should reduce the fish stock towards the $s(K)$ curve.

A sampling of possible trajectories is shown in the figure. Note that all trajectories eventually converge on a single long-run equilibrium point $(\underline{R},\underline{K})$ given in terms of recruitment and capacity (after depreciation and reinvestment). The equilibrium point corresponds to the point (x^*,K^*) in Clark et al. (1979) and represents the optimal equilibrium in the case where capital is perfectly malleable but not "abundant," so that the rental cost of capital must be included in variable costs. This is discussed in more detail in Charles (1982). Unlike in the Clark et al. (1979) model, this equilibrium point is not apparent from examining the $K = h(R)$ policy curve alone. In the continuous-time case, when a trajectory reaches the biomass level $x = x^*$, below (x^*,K^*), the optimal policy is an instantaneous investment to the capacity level $K = K^*$, thereafter remaining at (x^*,K^*). In discrete time, however, trajectories tend to "jump" across the line $R = \underline{R}$ rather than touch it smoothly, so the use of a single final impulse control at $\underline{R}$ is not a feasible method to reach the equilibrium point.

Furthermore, whereas equilibrium is reached in finite time with the continuous-time model, in a discrete-time situation the approach to equilibrium is asymptotic. The more gradual approach to equilibrium in discrete-time seems to reflect the benefit of incrementally increasing fleet capacity as the biomass grows, to take advantage of limited intraseasonal harvesting.

Apart from the differences mentioned above, the behavior of this instantaneous investment model and that of Clark et al. (1979) are quite similar, due to the pure compensatory nature of both the Beverton–Holt function and the continuous-time growth function used by Clark et al. (1979).

DELAYS IN INVESTMENT

As discussed under The Model, the introduction of delayed investment produces little change in the desired escapement and capacity in any given year, except inasmuch as payment for the new capacity must be made earlier than would be the case for instantaneous investment, and hence, effective capital costs are higher. However, management implications and the appearance of the optimal policies can differ substantially, since optimal capacity is now given as a function of escapement rather than recruitment.

Figure 2 depicts the optimal policies for the prawn fishery, with delayed investment, but otherwise unchanged parameters. With this fast-growing stock, a low escapement this year can still produce a large recruitment next year. Hence, it may be optimal to plan and pay for investment this year, even though stocks seem low, in the knowledge that when this new capacity becomes available next season, it can be used to harvest a much larger fish stock. This can lead to the situation shown in region R_4 of Fig. 2, where positive investment is optimal even though a harvesting moratorium is in place.

When such a situation arises, optimal escapement $s(K)$ must be independent of fleet capacity at low capital stocks (as

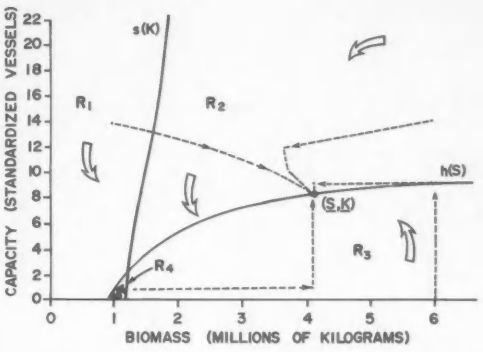

FIG. 2. Optimal policy functions for the base case prawn fishery with delayed investment. Sample trajectories and the long-run equilibrium $(\underline{S},\underline{K})$ are indicated.

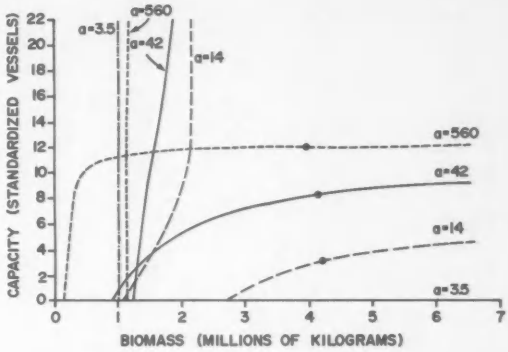

FIG. 3. Effect of prawn stock productivity on the optimal policy functions. Long-run equilibrium points for each parameter value are indicated.

in Fig. 2). Intuitively, the rationale for this is as follows. If, for a given escapement S, the current capacity is relatively low, investment will take place up to the capacity level $h(S)$, a level dependent solely on the escapement. Next year's recruitment, $F(S)$, is also dependent on the escapement. Hence, in such circumstances, current capacity is irrelevant to the determination of optimal escapement, which is therefore independent of K. Apart from this effect, however, the introduction of delays in investment does not change the $s(K)$ curve significantly.

It can be shown that for a fishery based on a slow-growing stock (whales), little qualitative change in the $h(S)$ curve is noticeable between the instantaneous and delayed investment cases; the policy curves in Fig. 1b remain essentially unchanged with the introduction of delays in investment.

In both the prawn and whale fisheries, trajectories again approach a long-run equilibrium, which can be compared with the corresponding open-access results obtained using equation 6. For the prawn fishery, the open-access capacity, 16.8 standardized vessels, is roughly double the optimal level. In the whale fishery, however, equilibrium biomass is very sensitive to the capital stock. Hence, the open-access and optimal capacities cannot differ by much; in fact, the values turn out to be very close, at 2505 and 2250 catcher days/yr, respectively. These results indicate that the extent to which open-access conditions produce overinvestment can vary considerably. Of course, the actual open-access investment behavior may be quite complicated, so that the present model only approximates the true situation.

PRODUCTIVITY AND CARRYING CAPACITY OF THE RESOURCE

Using the Beverton–Holt stock-recruitment function $R = F(S) = a^*S/(1 + a^*S/b)$, with $a^* = ae^{-mT}$, the maximum productivity (intrinsic growth rate) is $F'(0) = a^* = ae^{-mT}$. As a increases, holding the maximum recruitment b constant, recruitment becomes less and less dependent on escapement. One would expect that the higher the growth rate of the stock, the better off the fishery and hence the higher is the optimal capacity. This is confirmed for the prawn fishery in Fig. 3, where optimal policy functions are shown for each of $a = 3.5$, 14, 42, and 560, with $b = 7.0 \times 10^6$ fixed.

With $a = 3.5$, actual productivity is $ae^{-mT} = 0.95 < 1$, so the stock size will decline towards extinction even without fishing. Not surprisingly, a zero investment level is optimal in this case, but if for some reason fleet capacity is already in place, harvesting should occur down to the zero-profit level $s(K) = x_0 = 1.0 \times 10^6$. The optimal policy functions for the case $a = 14$ resemble those of the relatively low-productivity whale fishery, while $a = 42$ corresponds to the base case prawn fishery. As productivity increases, the $h(S)$ optimal capacity curve continues to shift upwards. The limiting case where recruitment is independent of escapement is approximated here by setting $a = 560$; the optimal capacity curve is fairly flat, with $h(S) \simeq 12$, for all but the lowest escapements.

The optimal escapement at low fleet capacity decreases steadily towards x_0 with a declining biomass growth rate. This confirms the idea that with a slow-growing stock and a low level of capacity one has little incentive to conserve the current stock, which will decline towards a low long-run equilibrium even without fishing. On the other hand, at high capacity levels K, the optimal escapement $s(K)$ depends on the intrinsic growth rate in a rather complicated way (see Fig. 5). As before, $s(K) \simeq x_0$ if productivity is very low, but with increases in the growth rate, the reproductive potential of the stock is improved, and higher escapements $s(K)$ are desirable. Ultimately, however, at high productivity, recruitment becomes less dependent on escapement, so that the optimal escapement $s(K)$ can be reduced, increasing immediate benefits with little effect on future stocks.

The maximum recruitment level, b, serves as a suitable indicator of the carrying capacity of a fish stock with Beverton–Holt dynamics. The value $b = 7.0 \times 10^6$, used in the base case, was derived from the sample mean of recent prawn recruitment data and has been substantially revised and extended from that used in the analysis of Clark and Kirkwood (1979): their older data produce the value $b = 11.3 \times 10^6$. The optimal policies based on each of these carrying capacity values, with productivity set at $a = 42.0$ in both cases, indicate that revising the data produces substantial movement in

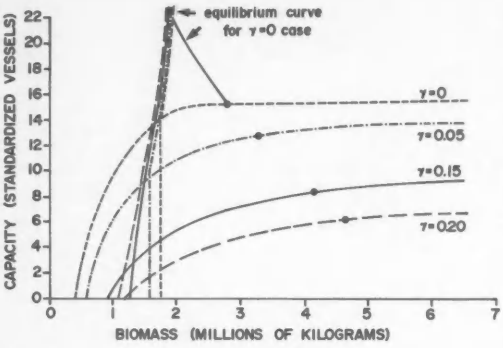

FIG. 4. Role of depreciation in the base case prawn fishery. The zero-depreciation equilibrium curve is shown, together with equilibrium points corresponding to $\gamma = 0.05$, 0.15, and 0.20.

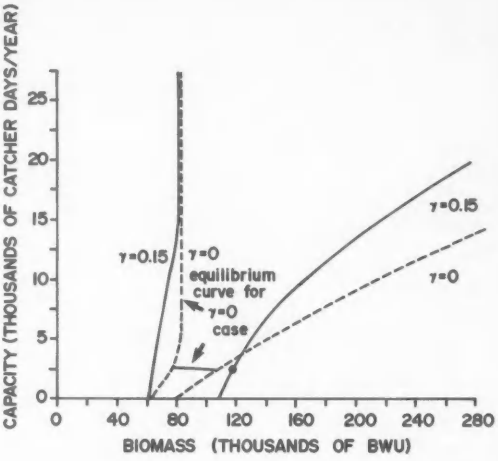

FIG. 5. Role of depreciation in the whale fishery. The equilibrium point for the $\gamma = 0.15$ case and the equilibrium curve for the $\gamma = 0$ case are shown.

both optimal policy functions. The optimal equilibrium capacity declined from 14.5 to 8.2 if the new data are used in place of the old (see Charles 1982 for further details). This relative decrease in optimal capacity holds also when higher values of productivity, such as $a = 560$, are considered.

In summary, the productivity and carrying capacity of the fish stock can have substantial effects on the optimal policies, in particular the optimal capacity function. This is especially of interest in such cases as the banana prawn fishery, where little is known about the stock-recruitment relationship. Dealing with parameter uncertainty in these fisheries becomes an important problem for further research.

DEPRECIATION RATE

The value of the depreciation parameter, $\gamma = 0.15$, used by Clark and Lamberson (1982) was utilized in the base case runs for both the prawn and the whaling fisheries. In a capital investment model, it is of interest to examine the effect on optimal policies of variations in the depreciation rate.

The results for the prawn fishery (Fig. 4) are intuitively appealing. A decrease in the depreciation rate leads to an upward shift in the investment curve $h(S)$, reflecting the increased life and hence the increased value of a new unit of capacity. On the other hand, an increase in γ increases the desire to use capacity before it depreciates, leading to a shift in the $s(K)$ curve to lower escapements. This latter shift is less pronounced at high capacity values, where capital is relatively abundant in the "near future" even for $\gamma = 0.20$.

With no depreciation ($\gamma = 0$), capacity K can never decrease. Charles (1982) showed that the set of points (S,K) that satisfy $S = \text{Max}\,[s(K),F(S)e^{-qTK}]$ and that lie above the curve $K = h(S)$ form an equilibrium curve upon which all trajectories will converge. However, in the particular case of Fig. 4, the optimal capacity curve $h(S)$ is very flat for sufficiently large escapement levels. Hence, if the capital stock is initially low, fishery dynamics will be such that investment will occur up to the equilibrium level $K \simeq 15$. Thereafter, the biomass will adjust so as to approach the long-run equilibrium point on the $\gamma = 0$ optimal capacity curve.

For the whale fishery (Fig. 5) the variation of the $s(K)$ curve with γ is qualitatively similar to that of the prawn fishery. In the $\gamma = 0$ case, the long-run equilibrium will again lie somewhere on the equilibrium curve, above the curve $K = h(S)$. However, the optimal capacity curve is now sufficiently steep that if the fishery has a low initial capital stock, a wide range of equilibrium points may be reached, depending on the initial recruitment value.

The unusual aspect of these whaling fishery results is the intersection of the $h(S)$ investment curves derived for the two levels of depreciation and, in particular, the fact that for sufficiently large biomass levels, the optimal capacity level is higher with depreciation than without. As described above, one might expect that if a unit of investment is profitable, given a relatively high depreciation rate, then that same unit of investment is even more desirable if it is longer lasting (in the absence of depreciation). Indeed, this is the case with the prawn fishery results above.

This counterintuitive result can be explained by considering the interaction of two key fishery parameters, the biological productivity (a or $a \cdot e^{-mT}$) and the relative cost of capital (δ/cT). The latter is a measure of the fishing fleet's capital intensity, being the ratio of unit capital costs, δ, to maximum yearly unit variable costs, $c \cdot T$ (the quantity δ/cT is discussed further in Charles 1983). For the prawn fishery, the effective intrinsic growth rate is $ae^{-mT} = 11.45$, while the cost ratio is $\delta/cT = 11.3$. In the whale fishery, $ae^{-mT} = 1.04$ and $\delta/cT = 2.0$. Hence, both the resource productivity and the relative cost of capital differ considerably between the two fisheries. Modifying the prawn fishery by simultaneously reducing the productivity to $a = 14$ ($ae^{-mT} = 3.82$) and reducing the capital cost so that $\delta/cT = 2.0$ (implying $\delta =$ \$0.0832 million), optimal policies qualitatively similar to those of the whale fishery are obtained (Fig. 6).

An analysis of trajectories for the policy functions of Fig. 6

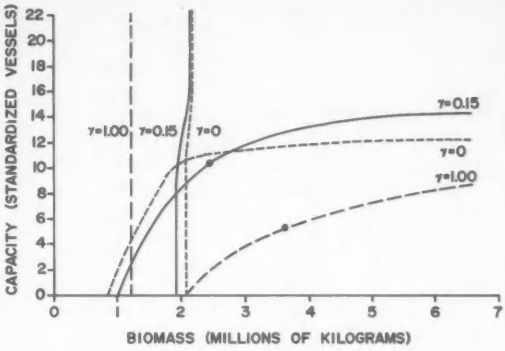

FIG. 6. Effect of the depreciation rate on a prawn fishery with low productivity ($a = 14$) and low capital cost (δ = A\$0.0832 million).

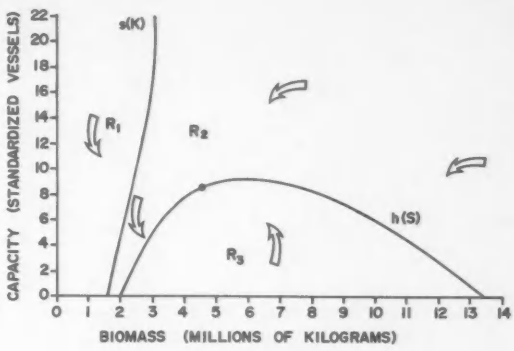

FIG. 7. Optimal policy functions for a fishery with a Ricker stock-recruitment function ($a = 11.639$, $b = 7.0 \times 10^6$) (see text for details).

indicates that the relative heights of the $\gamma = 0$ and $\gamma = 0.15$ optimal capacity curves are determined not simply by the ratio δ/cT but rather by a more complicated comparison of the present values of investment costs versus fishery rents. The zero-depreciation $h(S)$ curve represents a balance between investment costs and the natural preference for a larger capital stock to enable more rapid accumulation of rents as the stock is harvested down to equilibrium. Depreciation introduces two new factors: (i) the need for future investment to overcome depreciation and (ii) the desire to "beat" depreciation by harvesting the stock before the fleet "wears out." It is this latter effect that appears responsible for the $\gamma = 0.15$ $h(S)$ curve lying above the corresponding zero-depreciation curve at high escapement levels. However, as depreciation increases beyond 15%, the optimal capacity curve drops as the yearly costs of overcoming depreciation predominate. When $\gamma = 1.00$, so that vessels last for only one season, the $h(S)$ curve lies completely below its zero-depreciation counterpart. This rather complicated response to the depreciation rate seems to depend critically on actual parameter values, necessitating careful treatment of the data in specific applications. Nevertheless, an examination of the intrinsic growth rate, a, and the ratio of capital to operating costs, δ/cT, provides a useful indication of the role that depreciation might play in a particular fishery.

Capital Cost, Fish Price, and Discount Rate

This section summarizes results concerning the sensitivity of optimal investment and escapement levels to unit capital costs (relative to operating costs), selling price, and discount rate (for further details see Charles 1982).

The effects of changes in the unit capital cost, with unit variable cost (c) fixed, have been examined for the base case ($a = 42$) prawn fishery and for an $a = 14$ (lower productivity) prawn fishery. In the former case, a halving of the capital cost resulted in a 1.7-fold increase in equilibrium capacity. In the latter case, a reduction to almost one-sixth the usual capital cost, from \$0.47 million to \$0.0832 million, produces a 3.5-fold increase in the equilibrium capacity (with $\gamma = 0.15$). In both cases, optimal escapement at low capacities increased as capital cost decreased, reflecting the increased benefit in saving more of the fish stock for the future, at which time capacity will be higher.

The variation of the optimal policy functions with fish price was examined for the $a = 42$ prawn fishery. A doubling of the price, from its actual level of \$0.9·kg^{-1} to \$1.8·kg^{-1}, produced more than a doubling in equilibrium capacity, while a halving of the price made investment entirely uneconomic, so that depreciation slowly reduces the fleet size to zero. However, harvesting still takes place in this low-price case, as long as $R > s(K)$, although the escapement target $s(K)$ has increased relative to the base case.

Optimal policy functions have been obtained for the $a = 42$ prawn fishery with discount factors (and corresponding discount rates) of $\alpha = 0.99$ (1%), 0.90 (11%), and 0.8 (25%). Naturally, the lower the rate of discounting, the higher the benefit from investing in capacity for the future (to become available next year) and the higher the desired escapement, $s(K)$, to be left at the end of the current season. While optimal escapements (for fixed K) increase with α, the equilibrium escapement decreases with α, reflecting the optimality of using the increased capacity that becomes available with low discounting.

Ricker Stock-Recruitment

Results presented to this point have been based on the Beverton–Holt stock-recruitment function. In this section, these are compared with results obtained using the Ricker form, $R = F(S) = aS \cdot e^{-aS/eb}$, which has the property that recruitment attains a maximum value of $R = b$ at $S = eb/a$, and thereafter declines roughly exponentially. Since the fleet capacity target $K = h(S)$ is determined from the equation $V_K[F(S),K] = \delta/\alpha$, it would be expected that the optimal capacity will follow the qualitative behavior of the stock-recruitment function $F(S)$, in this case initially increasing to a maximum and thereafter decreasing.

Numerical results (Fig. 7) confirm this expectation. The parameters of the Ricker function used in this example, namely $a = 11.639$ and $b = 7.0 \times 10^6$, were chosen so that the maximum recruitment is identical to that of the

TABLE 2. Optimal value function $V(R,K)$ for the base case prawn fishery. Recruitment is given in millions of kilograms, capacity in standardized vessels, and value in millions of Australian dollars.

	Recruitment						
Capital	1.0	2.5	4.0	5.5	7.0	8.5	10.0
0	3.2	3.4	3.5	3.6	3.7	3.7	3.7
2	4.0	4.3	4.6	4.8	4.9	5.1	5.2
4	4.8	5.2	5.5	5.8	6.1	6.4	6.6
6	5.5	6.0	6.5	6.9	7.3	7.7	8.0
8	6.3	6.9	7.4	7.9	8.4	8.9	9.3
10	7.0	7.6	8.3	8.9	9.5	10.0	10.6
12	7.7	8.4	9.2	9.9	10.5	11.1	11.8
14	8.4	9.1	9.9	10.7	11.5	12.2	12.9
16	9.0	9.7	10.6	11.5	12.3	13.1	13.9
18	9.5	10.3	11.3	12.2	13.1	14.0	14.8
20	10.1	10.9	11.8	12.8	13.8	14.7	15.6
22	10.5	11.4	12.3	13.4	14.4	15.4	16.3
24	10.9	11.8	12.7	13.8	14.9	15.9	16.9
26	11.3	12.2	13.1	14.2	15.3	16.4	17.5
28	11.6	12.6	13.5	14.5	15.7	16.8	17.9

Beverton–Holt form for the base case prawn fishery and occurs at $S = 6.0 \times 10^6$ (to produce a reasonable agreement with the Beverton–Holt curve for low and medium escapements). Other model parameters are unchanged from those given in Table 1 for the prawn fishery, and delayed investment is assumed.

Note that there is a threshold level of escapement and hence of $F(S)$, below which no investment occurs. At high escapements, $F(S)$ decreases and eventually drops below its threshold value; thereafter, a zero fleet size would be preferred, at least temporarily (the horizontal scale in Fig. 7 has been changed from that used in previous results to include this upper cutoff). The optimal escapement curve $s(K)$ behaves similarly to those of Beverton–Holt cases, except the optimal high-capacity escapement has substantially increased, reflecting reduced productivity at low escapements for this particular Ricker curve.

VALUE FUNCTION

To this point the optimal policy functions, $s(K)$ and $h(S)$, have been derived and studied under various assumptions and parameter combinations. However, the dynamic programming approach produces not only the optimal policies but also the optimal value function. Indeed, for any policies $s(K)$ and $h(S)$, the corresponding value function is the solution of equation 1′, with $S^*(R,K)$ and $I^*(S^*,K)$ depending on $s(K)$ and $h(S^*)$ through equations 5 and 3, respectively.

A sample value function, corresponding to the optimal policy functions for the base case prawn fishery, is represented in Table 2, which shows that for Beverton–Holt population dynamics, $V_R > 0$, $V_K > 0$, $V_{RK} > 0$, and $V_{KK} < 0$ throughout the $R-K$ range considered; this is consistent with the assumptions made under Heuristic Analysis and Numerical Method. At low levels of the capital stock, the value function is quite insensitive to the level of recruitment, R. This reflects the fact that, with low fleet capacity, increased recruitment has little effect on rents for the current season, and since the prawn stock is fast-growing, differences in this year's stock size tend to substantially disappear by next year. At sufficiently high capacity levels, the target escapement can be attained from a wide range of recruitment values. For such (R,K) combinations, $V(R,K) = pR - (c/q) \log (R) + W(K)$, and hence, changes in V due to variations in R can be easily calculated within this range. Numerical results shown in Table 2 agree with such analytic calculations.

Discussion

In the instantaneous investment case, results obtained here correspond closely to those of Clark et al. (1979), the primary difference between the seasonal and continuous-time models being the more gradual approach to equilibrium in the discrete-time case. The important conclusion of Clark et al. (1979) regarding the optimality of "a complex pattern of expansion, overcapacity, and gradual contraction via depreciation" towards an "optimal sustained yield" equilibrium holds for the present seasonal model as well.

As in Clark et al. (1979), optimally managed fisheries will tend to move between three primary regimes: (i) a high-biomass, low-capacity regime, with both harvesting and investment being desirable, (ii) a high-biomass, high-capacity situation, in which investment is unwarranted but harvesting takes place, and (iii) a low-biomass case in which the fishery is essentially shut down, with neither harvesting nor investment being desired.

The introduction of delays in investment, as well as adding further realism to the model, produces the possibility of a fourth management regime in which the resource stock is too low to permit harvesting but is expected to recover during the "investment delay" period. In such cases, planning and payment for investment becomes desirable when current capacity is sufficiently low. Assuming linear variable costs and a present value rent maximization objective, optimal management was found to be characterized by capacity and escapement target curves, $h(S)$ and $s(K)$, representing the optimal capacity for given escapement S and the optimal escapement for a given fleet capacity K, respectively.

The optimal capacity function proved to be particularly sensitive to the fish price and stock-recruitment parameters, indicating the potential importance of including parameter uncertainty in the analysis. Variations in the depreciation rate produced rather complicated effects, depending both on the magnitude of unit capital costs (relative to operating costs and other economic factors) and on the actual values of the depreciation rate being considered. It was suggested that if capital is relatively inexpensive and if the depreciation rate is positive but not too large, the optimal capacity at high stock sizes can be greater with depreciation than without. This result runs counter to the usual idea that depreciation, as a type of fishing cost, should lead to lower investment. It appears to be caused by a dynamic disequilibrium incentive to harvest the resource quickly, before the fleet "wears out."

In the case of Ricker stock-recruitment, the optimal capacity function adopted an appearance mimicking that of the stock-recruitment curve itself, increasing rapidly at low escapements and declining relatively slowly at higher escapements. This effect is due to the delay in bringing new investment online; desired capacity for next season depends on the current escapement, acting through the reproduction function $F(S)$. This property can be used to predict roughly the qualitative appearance of optimal investment curves based on other stock-recruitment relationships.

While numerical results have been obtained here for two specific fisheries, the methodology and the qualitative results can be expected to apply in many fisheries, as well as in forestry and agricultural investment problems. It is clear from both qualitative and quantitative results presented here that a full analysis of renewable resource management must include questions of optimal investment strategies. Indeed, for many of the cases examined, the investment aspect is substantially more complex than the more widely studied optimal harvesting problem.

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I am grateful to my thesis supervisor, Colin W. Clark, and to Gordon R. Munro and Donald Ludwig for many stimulating discussions and considerable guidance throughout the course of the doctoral research on which this paper is based. Robert McKelvey and an anonymous referee have also provided helpful comments and suggestions. Finally, I would like to thank NSERC for financial assistance and the Institute of Applied Mathematics and Statistics at the University of British Columbia for providing a productive research environment.

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Optimal Fisheries Investment under Uncertainty

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CHARLES, A. T. 1983. Optimal fisheries investment under uncertainty. Can. J. Fish. Aquat. Sci. 40: 2080–2091.

A full analysis of optimal fisheries investment strategies must take into account high levels of uncertainty in future fishery returns, as well as irreversibility of investment in specialized, nonmalleable fishing fleets. A stochastic optimization model is analyzed using dynamic programming to determine optimal policy functions for both fleet investment and fish stock management within an uncertain environment. The resulting policies are qualitatively similar to those found in the corresponding deterministic case, but quantitative differences can be substantial. Simulation results show that optimal fleet capacity should be expected to fluctuate over a fairly wide range, induced by stochastic variations in the biomass. However, the performance of a linear-cost risk-neutral fishery is fairly insensitive to variations in investment and escapement policies around their optimum levels, so that economic optimization is "forgiving" within this context. A framework of balancing upside and downside investment risks is used here to explain the roles of several fishery parameters in relation to optimal investment under uncertainty. In particular, the intrinsic growth rate of the resource and the ratio of unit capital costs to unit operating costs are found to be key parameters in determining whether investment should be higher or lower under uncertainty.

CHARLES, A. T. 1983. Optimal fisheries investment under uncertainty. Can. J. Fish. Aquat. Sci. 40: 2080–2091.

Une analyse complète des stratégies d'investissement optimales dans les pêches doit tenir compte de la grande incertitude qui caractérise les revenus, de même que l'irréversibilité de l'investissement dans des flottilles de pêche spécialisées, non malléables. Nous analysons dans l'article qui suit un modèle d'optimisation stochastique à l'aide d'une programmation dynamique, afin de déterminer des fonctions de politiques optimales, tant pour l'investissement dans les flottilles que pour la gestion des stocks de poissons dans un climat d'incertitude. Les politiques qui en résultent sont qualitativement identiques à celles qui avaient été trouvées dans le cas déterministe correspondant, mais il peut exister d'importantes différences quantitatives. D'après les résultats de la simulation, on devrait s'attendre que la capacité optimale de la flottille varie dans une gamme assez étendue, par suite de variations stochastiques de la biomasse. Cependant, le rendement d'une pêche de coût linéaire et de risque neutre est assez insensible aux variations de l'investissement et aux politiques d'échappement autour de leurs niveaux optima, de sorte que l'optimisation économique est « indulgente » dans ce contexte. Afin d'expliquer les rôles de plusieurs paramètres de pêche par rapport à un investissement optimal dans des conditions d'incertitude, nous équilibrons les risques d'investissement en hausse et en baisse. Nous constatons, en particulier, que le taux de croissance intrinsèque de la ressource et le rapport entre coût en capital unitaire et coût d'opération unitaire sont les paramètres-clés quand il s'agit de déterminer si, dans des conditions d'incertitude, l'investissement devrait être plus élevé ou plus faible.

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THIS paper expands upon the fisheries investment model presented in Charles (1983a). That paper studied the comparative dynamics of optimal fisheries development in a deterministic world, where capital investment in the fishing fleet and harvest management ("investment in the resource") must be considered simultaneously. As in the related work of Clark et al. (1979), nonmalleable capital and the resulting irreversibility of investment played a key role.

In this paper, the irreversible investment problem is made even more realistic by recognizing that fisheries investment decisions must typically be made within an uncertain environment (c.f. Arrow and Lind 1970; Brock and Mirman 1978). In particular, uncertainty is incorporated here in the form of year-to-year stochastic resource fluctuations, so that future fish stock levels cannot be predicted in advance (although average values are known) — this is certainly a common

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feature of most fisheries. Primary emphasis is placed on examining (i) the appearance of an optimally managed stochastic fishery, (ii) the role of economic and ecological parameters in determining how uncertainty affects optimal investment, and (iii) the relative performance of deterministic and stochastic investment policies.

Questions of optimal fisheries investment under uncertainty have also been considered by Dudley and Waugh (1980) and McKelvey (1983). These interesting papers examine, respectively, the effects of several stochastic components in the optimal capacity problem and the optimal mix of "specialist" and "generalist" and "generalist" vessels in a fishing fleet. However, both studies make the simplifying assumption that the fish population in any year is independent of past harvests; this avoids the complexities involved in the joint investment problem but limits the number of fisheries to which their results can be applied. For recent reviews of other literature on fisheries management under uncertainty, see Andersen and Sutinen (1983) and Spulber (1983).

The Model

The stochastic model utilized here is a direct analog of the deterministic case discussed in Charles (1983a), to which the reader is referred for details. To summarize, the dynamics of the fish stock (R) and the fishing fleet (K) are given by

$$R_{n+1} = F(S_n)\cdot Z_n$$

$$K_{n+1} = (1 - \gamma)K_n + I_{n+1}$$

where in any year, the decision variables, escapement (S) and investment (I), are constrained by the current fish stock and capital stock, according to

$$R_n e^{-qTK_n} \leq S_n \leq R_n$$

$$I_{n+1} \geq 0.$$

The former reflects a constraint on intraseasonal harvesting effort, $0 \leq E(t) \leq K$, while the second constraint represents the irreversibility of investment. The lognormal random variables $\{Z_n\}$ are assumed to be independent and identically distributed, with mean value 1 ($E(Z_n) = 1$) and with log (Z_n) having variance σ^2. Hence, recruitment R follows a lognormal probability distribution with mean $F(S)$, where S is the previous year's escapement and $F(\cdot)$ is the corresponding deterministic stock-recruitment function.

Use of the lognormal distribution is motivated by two factors: (1) it is the natural distribution to reflect the large number of independent multiplicative effects facing the growth of fish from the egg to the adult stage and (2) it reproduces qualitative features of fisheries data, where one often sees a large number of low to medium recruitments and occasional very large recruitments.

As in most other fisheries management models, it is assumed that yearly recruitment and escapement are observable, although in practice, errors in measuring the biomass certainly add to the overall level of uncertainty and complicate the optimization analysis.

The resource management problem can be summarized as follows.

1) Given initial biomass R and capacity K, an optimal end-of-season escapement S^* must be determined.

2) The desired capacity for the following year is determined, and payment is made for the corresponding investment I^*. (A 1-yr delay in bringing new investment online is assumed, contributing to the resource manager's uncertainty in a stochastic environment.)

3) Harvesting and stochastic population dynamics occur, so that by the start of the next season, the biomass is $R' = F(S^*)\cdot Z$ for some value of the random variable Z.

4) Depreciation and investment take place, producing a capacity $K' = (1 - \gamma)K + I^*$ next season.

It is assumed throughout this paper that the social resource manager is risk neutral, that the fishery faces perfectly elastic demand (with given constant selling price p), and that costs are linear, with unit cost of harvesting effort c and unit cost of capital δ. (These assumptions will be relaxed in future work. Andersen (1982) and Pindyck (1982) considered questions of optimal harvesting under price variability but did not address the capital investment problem.)

The yearly rents accruing to the fleet, as a function of recruitment, capacity, escapement, and investment, are as in Charles 1983a:

$$\pi(R,K,S,I) = p(R - S) - (c/q)\log(R/S) - \delta I.$$

The fishery optimization problem is as before, with the exception that now, future fish stock sizes are averaged over a probability distribution. Specifically, next season's recruitment follows the lognormal density $R_{n+1} \sim \phi_{F(S),\sigma}(\cdot)$ with

$$\phi_{\bar{R},\sigma}(R) = [\sqrt{(2\pi)}\sigma R]^{-1} \times \exp\{-(\log R - \log \bar{R} + \sigma^2/2)^2/2\sigma^2\}$$

where $\bar{R}$ is the mean, given by $\bar{R} = F(S)$, and the variance is $\bar{R}^2(e^{\sigma^2} - 1)$.

The dynamic programming equation corresponding to this problem is then given by

$$(1)\quad V(R,K) = \max_{R\cdot\exp(-qTK)\leq S\leq R} \max_{I\geq 0} \{\pi(R,K,S,I) + \alpha E[V(R', (1 - \gamma)K + I)]\}$$

where (R,K) is the "state" this year, (S,I) are the controls (decision variables), R' is next year's recruitment (lognormally distributed as above), and α is the discount factor.

Heuristic Analysis and Numerical Method

Heuristic Analysis

Equation 1 is identical to the corresponding equation in Charles 1983a, except that the future value of the fishery is now averaged over possible future recruitments. The heuristic analysis proceeds in a similar manner, producing the following optimality results for the target investment curve $K = h(S)$, the target escapement curve $S = s(K)$, and the actual (feasible) investment and escapement, $I^*(S,K)$ and $S^*(R,K)$, respectively:

$$(2)\quad E\{V_K[R, (1 - \gamma)K + I]\} = \delta/\alpha \quad \text{or } I = 0 \text{ if } E\{V_K[R, (1 - \gamma)K]\} < \delta/\alpha$$

$$(3)\quad I^*(S,K) = \max[h(S) - (1 - \gamma)K, 0]$$

$$(4)\quad \alpha[F'(S)/F(S)] \cdot E\{R' \cdot V_R[R', (1-\gamma)K + I^*(S,K)]\} = p\left(1 - \frac{x_0}{S}\right)$$

$$(5)\quad S^*(R,K) = \begin{cases} R \cdot \exp(-qTK); & R > s(K)\exp(qTK) \\ s(K); & R \text{ intermediate} \\ R; & R < s(K) \end{cases}$$

where the expectation over R is with respect to the lognormal density $\phi_{F(S),\sigma}(\cdot)$, $x_0 = c/pq$ represents bionomic equilibrium, $K = h(S)$ solves $E_z\{V_K(F(S)\cdot Z,K)\} = \delta/\alpha$, and $S = s(K)$ solves equation 4.

In Charles (1982) it is shown that the resulting optimal policy curves are qualitatively similar to their deterministic analogues. The important questions, then, concern the behavior of optimally managed stochastic fisheries and the extent to which randomness affects the quantitative aspects of the optimal policies.

Numerical Method

Based on the above heuristic results, numerical methods have been developed to solve equation 1, using policy iteration to determine the optimal $S = s(K)$ and $K = h(S)$ curves. The approach used here extends methods of Ludwig (1979) and Ludwig and Walters (1982); it is more refined than that used in Charles (1983a), involving the evaluation of integrals over an infinite interval to determine the expected value function $E[V(\cdot,\cdot)]$.

The policy iteration algorithm proceeds as follows. First, an initial guess is made for the policy functions $s(K)$ and $h(S)$. For these functions, the value function $V(R,K)$ and its first partial derivatives V_R and V_K are determined simultaneously over all points on a discrete grid in the biomass/capacity plane. (An 8 by 8 grid was used in all cases, except for those involving the whale fishery (see Numerical Results below) where a 12 by 12 mesh was necessary to provide suitable accuracy.) The values of V, V_R, and V_K implicitly define a differentiable surface in $R-K$ space. It is necessary to extrapolate this surface beyond the limits of the discrete grid, so as to include the entire range of possible biomass values, $(0,\infty)$; the extrapolation procedures are described in Charles (1982). (In fact, for reasons of numerical accuracy and convenience, the method uses $x = \log(R)$ in place of R as a state variable. Naturally, this change of variables does not affect the final results.)

The next step is to improve upon the initial policy functions by inserting the newly found values of V, V_R, and V_K into the optimality equations 2 and 4. Solving these equations numerically produces new policies $s(K)$ and $h(S)$, which can be expected to outperform the policies used at the previous step. Repeating the process using these new policies results in an iterative approach to the overall optimum. This scheme performed well for all cases discussed below, although convergence was rather slow in the absence of depreciation ($\gamma = 0$).

Numerical Results

The numerical methods described above permit a full solution to the management problem of determining optimal investment and escapement policies within an uncertain environment. As in Charles (1983a), the Australian Gulf of Carpenteria banana prawn fishery (Clark and Kirkwood 1979) and the aggregated pelagic whaling fishery (Clark and Lamberson 1982) are examined. In most cases, the general form of the prawn fishery has been used as the primary source of data, but the parameters have been varied to study comparative dynamics. The base parameters for both fisheries are as in Charles (1983a). (Although the model used here is simple in comparison with most real-world fisheries, there is nothing specific in its structure to detract from fairly wide applicability. Hence, with suitable caveats, the general results should hold also in other fisheries and indeed other renewable resource industries.)

The underlying stock-recruitment function $F(S)$, representing the average recruitment for a given escapement S, is taken to be either $F(S) = aS/(1 + aS/b)$ or $F(S) = aS \cdot e^{-aS/eb}$ for the Beverton–Holt and Ricker cases, respectively. The parameters a and b then represent the maximum productivity and the maximum possible mean recruitment. For the prawn fishery, the fairly high, but realistic, value of $\sigma = 0.58$ was used in most cases for the uncertainty parameter (representing the standard deviation of the logarithm of recruitment). This value of σ is the maximum likelihood estimate obtained by fitting a lognormal distribution to prawn recruitment data from G. P. Kirkwood (C.S.I.R.O. Division of Fisheries, Cronulla, Australia, personal communication), with the mean value of the distribution simply equated to the sample mean of the data.

In general, the approach used here is to compare optimal policy functions for a fishery subject to a fairly high degree of uncertainty ($\sigma = 0.58$) with the corresponding optimal policies in the absence of uncertainty ($\sigma = 0$). The latter deterministic results correspond to those of Charles (1983a) but are obtained using the more accurate numerical method described above.

The first subsection below describes the appearance and behavior of an optimally managed stochastic fishery. The following subsections examine the effect of several key fishery parameters in determining the role of uncertainty in optimal fisheries management. In particular, it is of interest to study whether investment increases or decreases with increasing uncertainty, and how the outcome is affected by (i) the intrinsic biomass growth rate, (ii) the capital cost (relative to variable costs), (iii) the discount rate, and (iv) the depreciation rate.

Appearance and Behavior of a Stochastic Fishery

Figure 1a depicts the optimal investment and escapement policy functions $h(S)$ and $s(K)$ for the base case prawn fishery with $\sigma = 0.58$. As discussed in the previous section, the curve $h(S)$ represents the target fleet capacity for next season, given escapement S this year. In other words, it is desirable to purchase $I^*(S,K) = \text{Max}[h(S) - (1-\gamma)K, 0]$ in new fleet capacity, even though stochastic recruitment next season can only be predicted roughly (i.e. in the mean) at the time of ordering the investment. The $s(K)$ optimal escapement curve is entirely analogous to its deterministic counterpart.

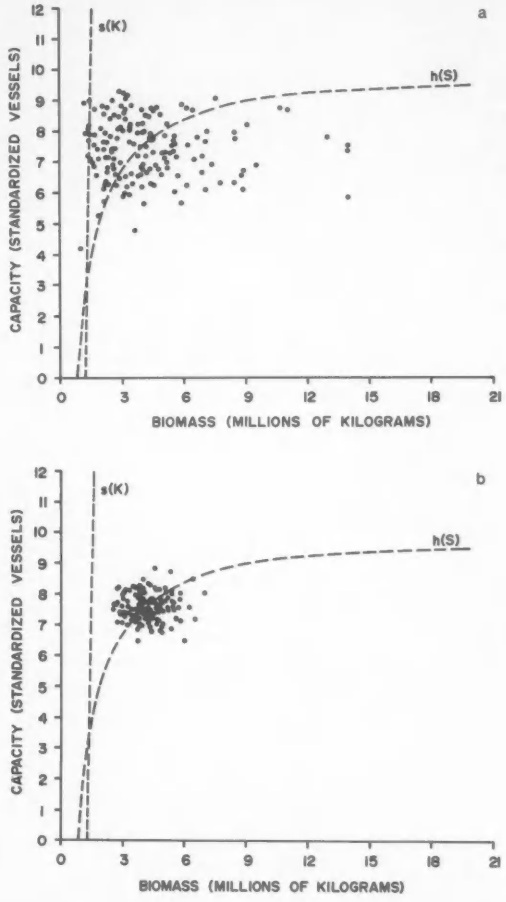

FIG. 1. Optimal policy functions, $h(S)$ and $s(K)$, for the stochastic base case prawn fishery, with uncertainty parameter (a) $\sigma = 0.58$ and (b) $\sigma = 0.2$. In addition, the steady-state distribution for this optimally managed fishery is approximated by the endpoints of 160 40-yr simulations, beginning each time at the quasi-equilibrium point (4.3×10^6, 7.75) (see text for details).

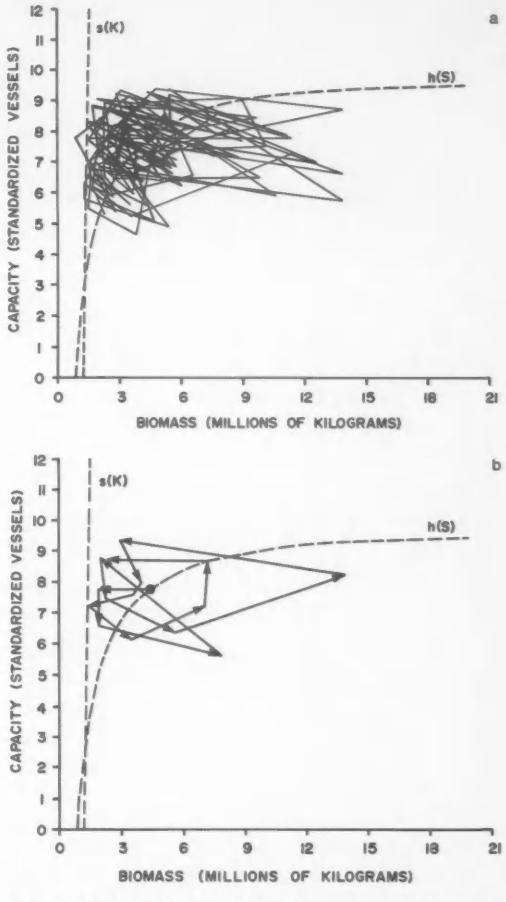

FIG. 2. Optimal policy functions for the base case stochastic prawn fishery, together with sample paths showing the effect of stochastic fluctuations on optimal management of the fishery. (a) Eight 20-yr sample paths, each beginning from the quasi-equilibrium point. (b) A single 15-yr outcome. The arrows join (S,K) points; the processes of depreciation, investment, recruitment, and harvesting occur successively between each pair of points.

The point $P(4.3 \times 10^6, 7.75)$ marked in Fig. 1a would be the long-run equilibrium point for the fishery if there were no random fluctuations. It is referred to here as a "quasi-equilibrium point"; deterministic fish and fleet dynamics tend to push the fishery towards this point, but stochastic perturbations prevent actual convergence. In fact, as pointed out by May et al. (1978) and Spulber (1983), deterministic equilibrium points translate into steady-state probability distributions in the stochastic case. In the present two-dimensional model, any steady state would also be two-dimensional, although the existence of such an equilibrium distribution has not been explicitly examined here. Instead, a steady-state distribution has been approximated by plotting the end points of a large number (160) of 40-yr fishery simulations, each emanating from the quasi-equilibrium point P; these end points are depicted in the figure.

The cloud of points shown in Fig. 1a can be interpreted as follows: the denser the points in a given region of the $S-K$ plane, the more likely is the fishery to lie in that region (i.e. to have that escapement and that capacity) over the long term. One can observe a considerable spread both in biomass and fleet capacity values about the quasi-equilibrium point. The spread in biomass values is due simply to the stochastic nature of the resource. Variation in the capital stock, on the

other hand, is an induced phenomenon; fluctuations in recruitment lead directly to variations in escapement, which in turn cause dispersion in fleet capacity, through the investment function $K^* = h(S)$. This effect will be even more pronounced with slower growing stocks, where particularly good or bad escapement levels will tend to influence the fishery for longer periods of time and will therefore have a greater effect on desired fleet capacity.

Since the resource is fairly fast-growing ($a = 42$), few points are found at low ($S < s(K)$) escapement levels. In fact, the distribution of points resembles a lognormal distribution in the S direction, truncated below at $S = s(K)$. This is unlikely to be the case precisely, however, since (S,K) rather than (R,K) points have been plotted, resulting in a tighter distribution. (Relatively high (lognormally distributed) R values are reduced by fishing pressure to comparatively low S values.) In addition, the spread of points in the S direction can be seen to be smaller at high capacities, since in this case fishing effort is sufficient to reduce even high recruitments down to escapements fairly near the $s(K)$ curve.

Figure 1b shows the effect of reducing the noise level from $\sigma = 0.58$ to $\sigma = 0.2$ in the prawn fishery. As expected, the steady-state distribution collapses to within a much smaller neighborhood of the quasi-equilibrium point. One would expect stochastic effects to be relatively unimportant at such low σ values; however, as shall be seen, the values of σ that can be considered "low" depend on the other fishery parameters. In the whale fishery, $\sigma = 0.2$ can be a substantial level of noise.

To illustrate more vividly the actual process of managing a fishery in a stochastic environment, Fig. 2a depicts a set of eight 20-yr sample paths for the optimally managed base case prawn fishery, with the recruitment chosen each year from a lognormal ($\sigma = 0.58$) density centred on $F(S)$, where S is the previous year's escapement. Lines are drawn joining successive (S,K) points, beginning at the quasi-equilibrium point. As above, it can be seen that optimal risk-neutral management results in considerable variation in the fleet capacity, as well as the biomass, over time.

Figure 2b shows this effect in more detail for a single 15-yr realization of the fishery's development. The four processes of (stochastic) recruitment, harvesting, investment, and depreciation combine to determine the movement from one (S,K) point to the next, governed by the policy curves. Since the resource is fast-growing and highly variable in this example, there is no apparent trend to return to the quasi-equilibrium point.

Cost of Capital

Consider Fig. 3 in which the optimal capacity curves $h(S)$ are shown for fisheries with $a = 14$ throughout, but with varying levels of uncertainty and unit capital cost.

To concentrate on the investment problem, the optimal escapement curves $s(K)$ have been omitted from Fig. 3. Typically, the optimal escapement level was found to be fairly insensitive to the degree of uncertainty for most of the parameter combinations considered, a result in accordance with those of other researchers. There are, however, cases where uncertainty does affect the $s(K)$ curves; these are discussed below. For the remainder of this paper, $s(K)$ curves are shown only in such cases.

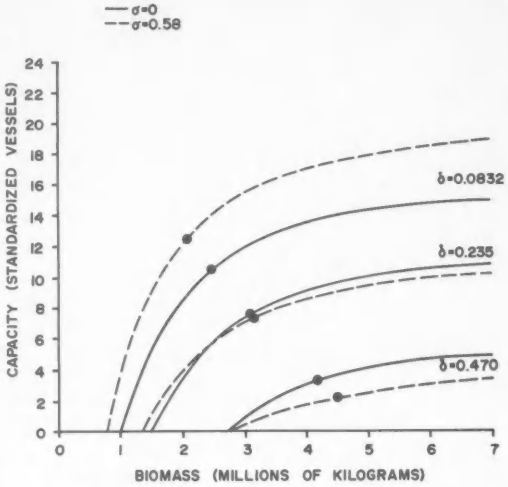

FIG. 3. Deterministic ($\sigma = 0$) and stochastic ($\sigma = 0.58$) optimal fleet capacity functions, $h(S)$, for an $a = 14$ prawn fishery with three values of the unit capital cost: $\delta = 0.0832$, 0.235, and 0.470 (Australian dollars $\times 10^6$).

It was seen in the deterministic analysis of Charles (1983a) that the important cost parameter in the investment problem is neither capital cost nor operating cost alone, but rather the ratio of the two. Specifically, a useful quantity to study appears to be δ/cT, the ratio of unit capital cost to maximum yearly operating cost (per unit of capital). In a sense, this measures the capital intensity of the fleet, since the present value of maximum total costs per unit of capacity is

$$\delta + \beta cT = cT[(\delta/cT) + \beta]$$

where

$$\beta = \alpha \sum_{n=0}^{\infty} [\alpha(1 - \gamma)]^n = \frac{\alpha}{1 - \alpha(1 - \gamma)}$$

allows for discounting and depreciation. The important aspect of Fig. 3 is the relative position of the $h(S)$ curve between the deterministic and stochastic cases, as this ratio of costs, δ/cT, varies. However, since cT is fixed here, it suffices to speak in terms of changes in the capital cost. It can be seen that at low capital costs, the optimal capacity is substantially higher in a fluctuating environment, while at high unit capital costs the reverse is true. At the intermediate level $\delta = \$0.235$ million, the optimal $h(S)$ curves in the deterministic and stochastic cases exhibit a crossover, so that the introduction of randomness increases optimal investment at low biomass levels while decreasing investment at higher stock sizes.

With regard to the $s(K)$ policy functions, the slower growing $a = 14$ fishery shows a slightly greater effect of randomness on the optimal escapement levels than in the $a = 42$ base case: this is indicated for the $a = 14$, $\delta =$

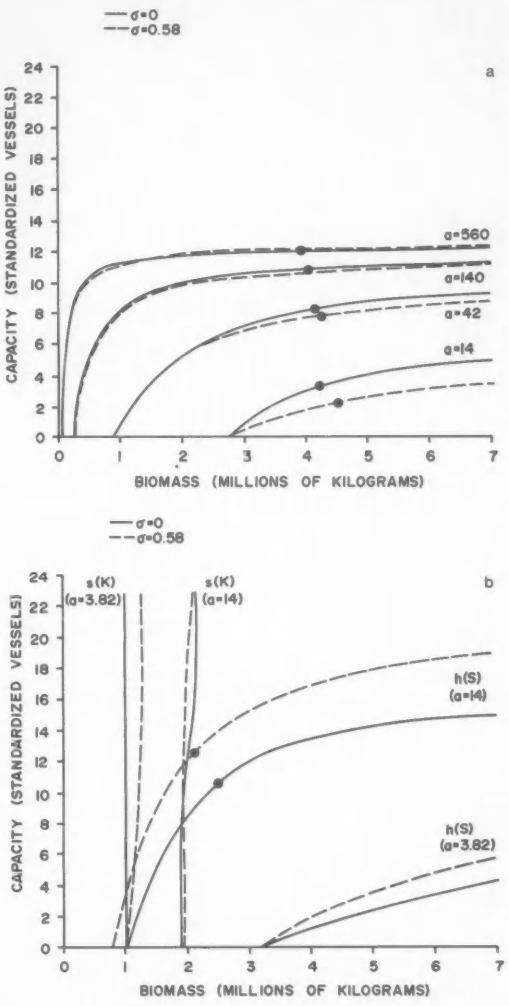

FIG. 4. Joint effect of the biomass growth rate and the level of uncertainty. (a) Optimal capacity functions for the base case prawn fishery with growth rates $a = 14, 42, 140$, and 560. (b) A lower level of unit capital cost is assumed (δ = A\$0.0832 million rather than δ = A\$0.47 million), and the values $a = 3.82$ and $a = 14$ have been used as possible fish productivity levels. In each case, deterministic ($\sigma = 0$) and stochastic ($\sigma = 0.58$) cases are considered.

\$0.0832 $\times 10^6$ fishery in Fig. 4b and is discussed further below.

The optimal capacity results described here can be explained by considering two opposing effects: the "downside" risk of suffering idle excess capacity in bad years and the "upside" risk of lacking sufficient capacity to take full advantage of good years. Ceteris paribus, the optimal fleet capacity increases with uncertainty given a relatively low ratio of capital to operating costs, since the balance of these risks is tilted towards the upside benefits of investing in extra capacity (at relatively low cost) to take advantage of exceptionally high recruitments. However, if unit capital costs are sufficiently high relative to operating costs, investment will decrease with the level of uncertainty. In this case the downside risk of more frequent bad years (when there is little or no return on the expensive investment) outweighs the advantages of having extra capital available to profit from good years. In the intermediate case, it appears that the role of uncertainty depends on the escapement level; at high biomass levels, the variance in recruitment is also high, so that the downside problem predominates. Hence, investment is lower under uncertainty; the balancing act tilts towards caution in investment. On the other hand, if escapement is already relatively low, and the stock tends (in the mean) to grow reasonably rapidly, then the potential benefits to extra (upside) investment outweigh the downside risk. Increased investment under uncertainty becomes optimal. Where the crossover will occur, if it does at all, seems rather difficult to predict. Indeed in most cases where a crossover appears, the difference between the $h(S)$ curves in the deterministic and stochastic cases tends to be small.

PRODUCTIVITY OF THE RESOURCE STOCK

Figure 4a shows deterministic and stochastic optimal capacity functions for each of four possible values of the growth rate parameter, with other parameters as in the base case fishery. (The value $a = 560$, corresponding to very high fish stock productivity, was chosen to approximate a situation of independence between recruitment and escapement.)

Examining Fig. 4a, one can observe a uniform progression from high to low growth rates. When $a = \infty$, it is shown in Charles (1982) that the optimal capacity must increase with the level of uncertainty. The case $a = 560$ follows this result, at least for reasonably high stock levels. However, with $a = 140$, the optimal capacity is lower under uncertainty, and this effect increases as the growth rate is decreased to $a = 42$ and then to $a = 14$. (As before, in each case, there is little difference between the stochastic and deterministic optimal escapements.)

If the unit capital cost is reduced, in this case to \$0.0832 million, results remain qualitatively unchanged. Figure 4b shows the deterministic ($\sigma = 0$) and stochastic ($\sigma = 0.58$) optimal policy functions for the two cases $a = 14$ and $a = 3.82$, with the lower capital cost. When natural mortality is taken into account the latter a value corresponds to a maximum net growth rate of 4% annually. This value was chosen to equal that of the whale fishery for comparative purposes discussed below. In this case, even at such low productivity, the unit capital cost is sufficiently low that the optimal capacity remains positive for both the deterministic and stochastic cases. The target capacity is generally higher under uncertainty, although there is a very slight crossover in the $h(S)$ curves, a result that is returned to in discussion of the whale fishery. As the biomass growth rate is increased to $a = 14$, the optimal capacity under uncertainty rises even further above its deterministic counterpart.

While optimal policies can be determined for the $a = 3.82$

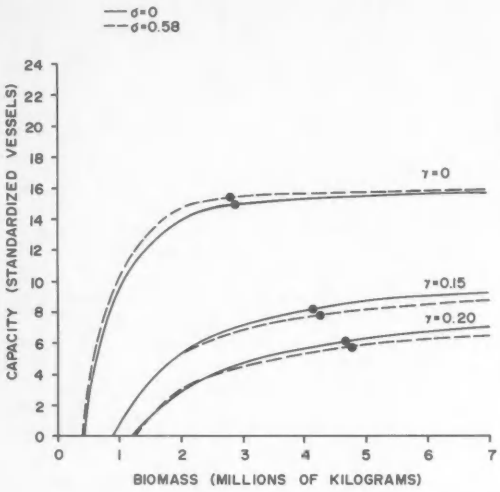

FIG. 5. Depreciation rate and uncertainty: the optimal deterministic and stochastic capacity functions are shown for each of $\gamma = 0$, 0.15, and 0.20 for the base case prawn fishery.

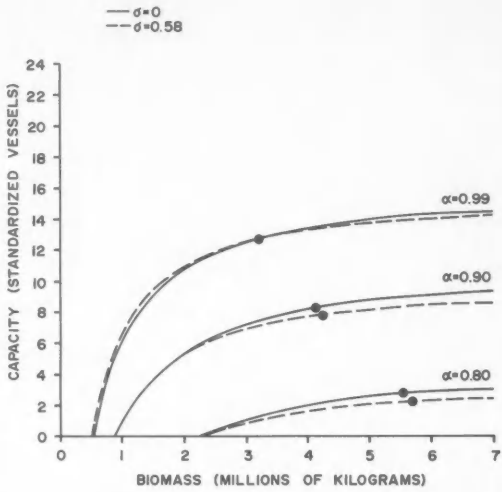

FIG. 6. Discount rate and uncertainty: the optimal deterministic and stochastic capacity functions are shown for each of $\alpha = 0.80$, 0.90, and 0.99 (with respective discount rates $r = 25$, 11, and 1%) in the case of the prawn fishery.

fishery, in fact this fishery would not be economically sustainable in a deterministic environment; the equilibrium occurs at $R = S = 0.28 \times 10^6$, which is well below the rent-dissipating bionomic equilibrium. Hence, no fishery will exist unless the resource is artificially enhanced or economic conditions improve significantly. In the stochastic case, however, there is always a possibility of the biomass reaching a sufficiently high level to warrant investment in the fishery. In the example shown here, such an occurrence will be rare, but amongst the world's fisheries, there will likely be many that should follow such a pattern of periodic development, responding to occasional exceptionally high recruitments.

With regard to optimal escapement levels, when $a = 3.82$, $s(K) = x_0$ (for all K) in the deterministic case, while $s(K) > x_0$ for $\sigma = 0.58$. Hence, the fishery should be driven towards its zero-profit level in the deterministic case but should be somewhat more conservationist under uncertainty. The $a = 14$ optimal escapement curves exhibit rather complicated behavior, with the stochastic $s(K)$ curve lying above the $\sigma = 0$ curve, except in an intermediate range of fleet sizes (between $K = 10$ and $K = 24$). This intermediate phase appears to be caused by the considerable variation in the fleet investment policies with uncertainty, since $I^*(S,K)$ enters into the optimality equation determining $s(K)$. However, in any case, the maximum difference between the $s(K)$ curves with $a = 14$ is only $\Delta s = 0.15 \times 10^6$.

The interplay between the degree of randomness and the intrinsic growth rate can be explained by appealing to the downside versus upside argument discussed above. The slower growing the resource stock, the greater the connection between recruitment and the previous season's escapement. In effect, the memory of the system is longer, so that both particularly low and particularly high recruitments will tend to be more persistent in the fishery (although, of course, stochastic fluctuations will cause deviations from this trend). This has implications for both the downside and upside aspects of the investment question. On the one hand, the downside risk for new investment will be greater, since the probability of suffering a series of bad years is increased. On the other hand, the upside benefits of extra fleet capacity are reduced because a high recruitment will tend to persist over several seasons and can therefore be harvested at a more leisurely pace, using less capital. The situation is reversed with fast-growing stocks, where the high recruitments produced in good years must be utilized immediately or forever lost, and hence, there is a strong incentive to invest in additional capital. On balance, therefore, the stochastic optimal capacity will always exceed its deterministic counterpart if recruitment is independent of past escapements ($a = \infty$), but this effect will decrease and likely reverse itself as the intrinsic growth rate decreases and downside risks begin to outweigh upside benefits. In fact, the effect of uncertainty on optimal investment depends jointly on the biomass growth rate and the capital cost to operating cost ratio (see Discussion for a consideration of this rather complex interaction).

DEPRECIATION RATE

Figure 5 shows that in the $a = 42$ case, the depreciation rate plays a role similar to that of the unit capital cost described above. In the absence of depreciation ($\gamma = 0$), the optimal capacity under uncertainty exceeds that of the deterministic case. Since capital is infinitely long-lived in such a case, the effective yearly rental cost of capital is relatively low. Hence, the downside risk of an increased capital stock (above that of the deterministic case) is relatively small. As the rate of depreciation increases, the effective time horizon

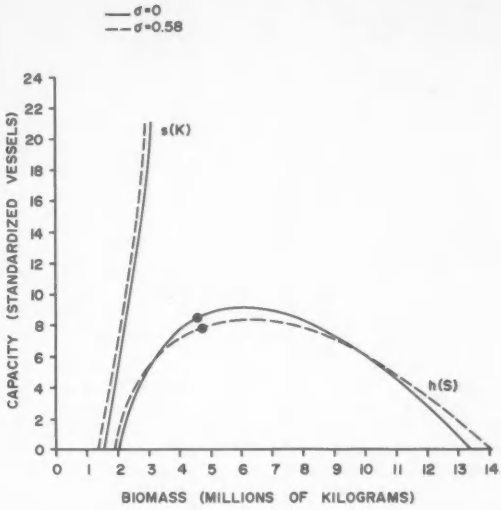

FIG. 7. Ricker stock-recruitment and uncertainty: the optimal policy functions $h(S)$ and $s(K)$ are shown for the prawn fishery with Ricker parameters $a = 3.172$ and $b = 7.0 \times 10^6$ and uncertainty $\sigma = 0$ and 0.58.

for a given unit capacity is shortened, so that the upside benefits of an extra unit of capacity are reduced, since there are likely to be fewer years in which the fishery could take advantage of a higher level of capacity. Thus, it is not surprising to see that the optimal capacity decreases with uncertainty when $\gamma = 0.15$ or $\gamma = 0.20$. In each of the three cases, the optimal escapement was insensitive to the level of uncertainty.

The effect of depreciation on this base case fishery can be summarized as follows: the lower the depreciation rate, the more likely is investment to be higher under uncertainty. In other words, the difference: "(stochastic optimal capacity) − (deterministic optimal capacity)" increases as the depreciation rate decreases.

With a slower growing ($a = 14$), lower capital cost ($\delta = \$0.0832$ million, $\delta/cT = 2.0$) fishery, Charles (1983a) found that for the deterministic case, optimal investment levels can actually increase with the depreciation rate, if escapement is sufficiently high. It was argued that this result was due to an incentive to harvest the resource quickly, before the fleet depreciates. If capital is relatively cheap, this incentive outweighs the effective increase in unit capital cost due to depreciation. However, the same behavior does not carry over to the corresponding stochastic fishery; results obtained for this fishery indicate that although investment levels are uniformly higher with $\sigma = 0.58$ than in the deterministic case, optimal capacity decreases as the depreciation rate is increased, for all escapement values. This may be due to one of two reasons: either (i) the stochastic investment levels are already sufficiently high that the resource can be harvested as rapidly as necessary or (ii) stochastic fluctuations add sufficient unpredictability to the fishery that extra investment, to allow more rapid harvesting in the face of depreciation, is not warranted.

DISCOUNT RATE

Figure 6 depicts the optimal policy curves with and without randomness for three values of the discount factor (α) and corresponding discount rate ($r = [(1 - \alpha)/\alpha] \times 100\%$).

It can be noted that as the rate of discounting is increased (i) the relative difference between optimal deterministic and stochastic fleet capacities increases and (ii) the region of escapements for which optimal investment is greater under uncertainty diminishes. Hence, an increase in the discount rate is similar in effect to an increase in unit capital cost, increasing the downside risk of investment under uncertainty. However, it appears that at least for these parameter combinations, there is relatively little interplay between the discount rate and the level of uncertainty over a broad range of discount rates. Of course, the role of the discount rate in resource management is a complicated one, and these limited results should not be extrapolated too far.

RICKER STOCK-RECRUITMENT

If Beverton−Holt population dynamics are replaced by a Ricker reproduction function, the heuristic analysis and the results of Charles (1983a) suggest that the optimal investment curve $h(S)$ should mimic the Ricker form, rising to a peak and then declining to zero. Figure 7 confirms this qualitative behavior. In terms of the future of the fish stock, very large escapements are as bad as very small ones; if $K = 0$ but escapement $S > 14.0 \times 10^6$, the expected value of a unit of investment is less than its cost and, hence, $I^* = 0$.

Comparing the deterministic and stochastic optimal capacity functions, one can see that if the future of the fish stock is relatively bright ($3.25 \times 10^6 < S < 9.75 \times 10^6$), then investment is lower under uncertainty, while the reverse is true if expected future stock sizes are relatively small. This is equivalent to a single crossover in the $h(S)$ curves for the Beverton−Holt case (e.g. compare the $a = 14$, $\delta = \$0.235 \times 10^6$ results shown in Fig. 3) and can be explained by examining the interaction of upside and downside investment risks, as above.

WHALE FISHERY

To this point, various modifications of the prawn fishery data have been considered. To check the robustness of the above results, this subsection examines the effect of uncertainty on the base case whaling fishery. Figure 8 shows the optimal capacity and escapement curves for the cases of $\sigma = 0$, $\sigma = 0.1$, and $\sigma = 0.2$, which cover a reasonable range of uncertainty for the aggregated Antarctic whale stocks.

It can be seen that increasing the level of uncertainty decreases the optimal capacity for relatively low stock sizes ($S < 360 \times 10^3$) but increases optimal capacity for high biomass levels. In other words, investment under uncertainty should respond to the state of the fishery; if the whale stock is particularly abundant, it is worthwhile taking advantage of

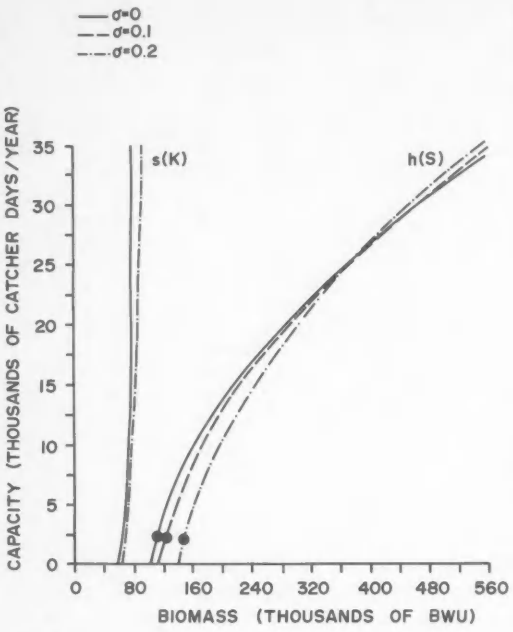

FIG. 8. Whale fishery under uncertainty: the optimal policy functions $h(S)$ and $s(K)$ are shown for each of the uncertainty levels $\sigma = 0$, 0.1, and 0.2 (the $s(K)$ curves for $\sigma = 0$ and $\sigma = 0.1$ differ negligibly).

good years by investing in capacity above the deterministic level, while for lower stock sizes a more conservative investment policy is to be preferred. However, the deterministic equilibrium point for the whale fishery lies at a low biomass level ($S = 114 \times 10^3$, $K = 2250$), so that even with $\sigma = 0.2$ one will rarely see escapements $S > 360 \times 10^3$ in the long term. On the other hand, the unexploited deterministic equilibrium lies at $S = 462 \times 10^3$. Hence, for this example the optimal stochastic policy faced with a virgin stock calls for an initial investment above the deterministic optimum but an investment level that is generally lower under uncertainty in the long term.

Note that both the maximum biomass growth rate and the ratio of unit capital cost to maximum yearly variable cost ($\delta/cT = 2.0$) for the base case whale fishery are the same as for a "prawn fishery" with $a = 3.82$ and $\delta = \$0.0832$ million (by design). Comparing Fig. 8 with results for that particular prawn fishery case, shown in Fig. 4b, one can see that the optimal policy curves are similarly behaved in most respects. In both cases, there is a crossover between the deterministic and stochastic $h(S)$ curves. However, the location and extent of the crossover is quite different in the two fisheries, possibly due either to the difference in noise levels being considered or to the difference in carrying capacity between the two fisheries. The latter, represented by the solution of $F(S) = S$, is relatively large for the whale fishery ($462 \times 10^3 \gg x_0 = 55 \times 10^3$) but small for the prawn fishery described above

TABLE 1. Expected value function $V(R,K)$ evaluated in a $\sigma = 0.58$ stochastic environment, using the appropriate optimal stochastic policies. Recruitment is given in millions of kilograms, capacity K in standardized vessels, and value in millions of Australian dollars.

	Recruitment							
Capacity	0.20	0.50	1.00	2.00	3.00	4.50	7.00	20.0
0.0	4.61	5.04	5.39	5.91	6.26	6.60	6.92	7.43
3.0	4.75	5.21	5.60	6.12	6.58	7.10	7.74	9.81
6.0	4.89	5.38	5.81	6.33	6.85	7.53	8.44	11.9
9.0	5.02	5.54	6.01	6.54	7.08	7.90	9.04	13.7
12.0	5.15	5.69	6.19	6.76	7.29	8.21	9.55	15.3
15.0	5.27	5.84	6.36	6.97	7.50	8.48	9.99	16.6
18.0	5.39	5.98	6.53	7.17	7.70	8.69	10.4	17.8
21.0	5.50	6.12	6.68	7.35	7.88	8.87	10.7	18.8

($0.28 \times 10^6 < x_0 = 1.0 \times 10^6$). In any case, the general result appears to be that investment is either zero or decreases with uncertainty for most reasonable escapement values in these fisheries; only in the case of exceptionally high escapements is it optimal for society to invest in fleet capacity above the deterministic optimum (assuming a low unit capital cost).

The primary difference between these results and others presented above is the fact that the crossover in $h(S)$ curves has been reversed, so that investment is now higher under uncertainty at high resource stock levels. The opposite effect was explained above by noting that biomass fluctuations, and the resulting downside risk, are relatively more important with large fish stocks. However, these results indicate that the explanation depends on resource productivity; when the fish population is very slow-growing, with a long inherent "memory," the downside risk diminishes as the stock grows, so that for an abundant stock, the optimal fleet capacity may actually increase with the level of fluctuations.

The optimal escapement for the whale fishery rises with increasing uncertainty, as in the $a = 3.82$, $\delta = \$0.0832 \times 10^6$ prawn fishery discussed above. It appears that management of a very slowly growing fishery should be more conservationist the higher the level of uncertainty; this is in accordance with previous research results. However, the difference between the $\sigma = 0$ and $\sigma = 0.2$ $s(K)$ curves is never very great, particularly for K values near the quasi-equilibrium point.

A particularly interesting result in comparing the deterministic and stochastic whale fisheries is the location of these quasi-equilibrium points: when $\sigma = 0.2$, the whale stock steady state is centered on $S = 148 \times 10^3$, 30% higher than the deterministic equilibrium. Hence, although there is little change in the $s(K)$ curves, use of the optimal stochastic policy can effectively lead to a substantially larger stock of whales (on average), while decreasing the (mean) optimal capacity by only 11%, from $K = 2250$ to $K = 2000$.

PERFORMANCE OF OPTIMAL AND SUBOPTIMAL POLICIES

At this point, two fundamental questions need to be addressed: how sensitive is the value of the fishery to changes in the policy curves $s(K)$ and $h(S)$ away from their optimum positions and how well do the policy functions obtained as

optimal for a deterministic environment compare with "true" optimal policies for the stochastic fishery?

The very nature of optimal controls suggests that small variations in the controls should have even smaller effects on the value function (Ludwig 1980). This indeed appears to be the case in the present model. Using the deterministic version, the optimal policy function $h(S)$ for the base case prawn fishery was perturbed first upwards and then downwards by 10%. The reduction in the value function was approximately 1.0% in both cases, a result in agreement with Ludwig's point that the variation in the value function should be proportional to the square of the fractional deviation in the policies.

It was shown above that policies that take into account fluctuations in the fishery's environment can differ from their deterministic counterparts by as much as 30−40%, for reasonable parameter combinations. Optimal fisheries investment, then, can be significantly higher, or significantly lower, under uncertainty. However, the optimal value function appears to be rather insensitive to changes in the policy functions away from their optimal positions. Table 1 gives the value function (at discrete grid points in the $R-K$ plane) based on the optimal policies for a stochastic ($\sigma = 0.58$) fishery with $a = 14$ and $\delta = \$0.0832 \times 10^6$. Table 2 represents comparable results using the optimal policies for the corresponding deterministic fishery, but evaluated in a stochastic ($\sigma = 0.58$) environment.

By comparing these value functions point by point, one can see that the loss from using the deterministic policy is never more than $0.2 million. For example, with $S = 2.1 \times 10^6$ (the quasi-equilibrium escapement) and $K = 0$, the optimal investment for the stochastic fishery is $I^* = 12.5$, while the deterministic policy produces $I = 8.8$, a 30% underinvestment. However, the reduction in value of the fishery caused by using the deterministic policy is roughly 3%, or only $0.16 million, a rather negligible amount when one considers the overall lack of precision attainable in real-world fisheries. Hence, while effects of uncertainty on the policies themselves can be considerable, the use of "incorrect" deterministic policies may not reduce the fishery's value significantly. The implications of this result are discussed below.

TABLE 2. Expected value function $V(R,K)$ for a $\sigma = 0.58$ stochastic environment, but determined using the "optimal" policies for the corresponding deterministic fishery. Recruitment is given in millions of kilograms, capacity K in standardized vessels, and value in millions of Australian dollars.

	Recruitment							
Capacity	0.20	0.50	1.00	2.00	3.00	4.50	7.00	20.0
0.0	4.48	4.90	5.23	5.75	6.11	6.45	6.76	7.25
3.0	4.63	5.08	5.46	5.96	6.43	6.95	7.57	9.63
6.0	4.77	5.25	5.68	6.18	6.69	7.38	8.28	11.7
9.0	4.90	5.41	5.87	6.39	6.93	7.75	8.88	13.5
12.0	5.03	5.57	6.06	6.62	7.15	8.06	9.40	15.1
15.0	5.16	5.72	6.24	6.84	7.37	8.35	9.84	16.4
18.0	5.28	5.87	6.41	7.05	7.58	8.57	10.2	17.6
21.0	5.40	6.01	6.58	7.24	7.77	8.76	10.6	18.7

Discussion

Results obtained here point to three primary conclusions, involving (i) the qualitative differences between deterministic and stochastic fisheries, (ii) the upside/downside determinants of optimal investment under uncertainty, and (iii) the performance of deterministic versus stochastic strategies. On the first point, heuristic analysis of the stochastic model indicated that optimal investment and escapement policies under uncertainty should not differ qualitatively from the deterministic case; numerical results confirmed this expectation. However, by simulating stochastic sample paths and steady-state distributions, it was shown that in practice the appearance of an optimally managed stochastic fishery is quite different from that of its deterministic counterpart. Even in the long run, optimal fleet capacity in a stochastic environment should be expected to fluctuate over a fairly wide range. This range will be greater the slower growing and the more variable the resource stock. In particular, an optimal investment program should allow the capital stock to respond positively to unusually "good" years, either by permitting increased entry of vessels or by direct acquisition of extra capital. This is done in full knowledge that idle capacity will then be greater in the "bad" years. (The possibility of various "political" pressures leading to the overutilization of this new capacity may be a real danger but has not been included in the model discussed here.)

The balancing of upside and downside risks has been used here to explain the quantitative effects of uncertainty on optimal fleet capacity. In all fisheries investment decisions there exists an upside risk of foregone benefits in exceptionally good years and a downside risk of suffering idle capacity in bad years. Whether optimal investment will be higher or lower under uncertainty (compared with the deterministic optimum) depends on the relative importance of these risks, which in turn are influenced by fishery parameter values.

The intrinsic biomass growth rate (a) and the ratio δ/cT of unit capital cost to maximum yearly operating costs are of particular importance in this regard. Figure 9 shows (a,δ) combinations (with cT fixed) for which optimal capacity is generally higher (+) or lower (−) under uncertainty ($\sigma = 0.58$), together with a rough curve dividing the two regions.

In general, investment will be higher under uncertainty if the resource is fast-growing and capital is relatively cheap. In this case, the upside benefits of extra fleet capacity are substantial, while the downside risks of idle capacity are not as critical. The reverse will be true for a slow-growing stock with expensive capital. This suggests a guiding principle for estimating the qualitative effect of randomness, without undertaking a full stochastic analysis: if the ratio of unit capital cost to yearly operating costs seems fairly low, and if the resource is reasonably fast-growing (as with prawn stocks), then investment is likely to be at least somewhat higher under uncertainty. This information may be useful in determining whether a fishery has indeed experienced overinvestment, or whether apparent excess capacity is in fact optimal given the history of the fishery's development in the face of uncertain future stock sizes.

The framework of upside versus downside fisheries investment risks is useful as well in analyzing the effects of other model parameters. It was found in particular that lower depreciation rates and lower discount rates increase the

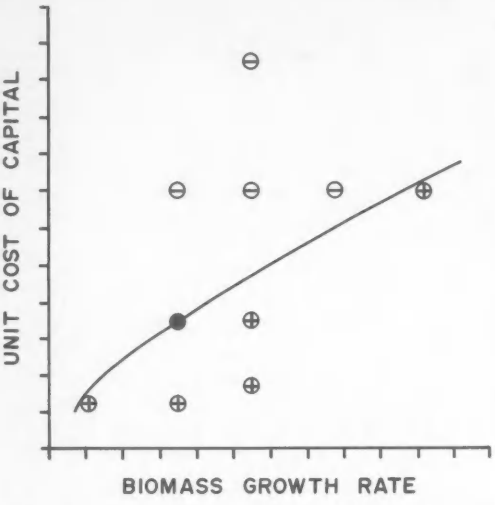

FIG. 9. Interaction between biomass growth rate and cost of capital in determining the role of uncertainty in fisheries investment. Points shown represent (a,δ) combinations that have been considered. Investment increases (+) or decreases (−) with uncertainty. (Diagram is not drawn to scale and the dividing line is approximate.)

tendency for optimal investment to be higher under uncertainty. In addition, the general results discussed above were found to apply to the case of Ricker stock-recruitment and to the aggregated whaling fishery.

Examination of the quantitative results obtained here shows that for moderate levels of variability, and reasonable parameter combinations, the relative difference between stochastic and deterministic optimal fleet capacities can reach 30−40%. This results in substantial over- or under-investment in fleet capacity when the deterministic model is used in place of a full stochastic model. Target escapements, on the other hand, tended to be remarkably insensitive to the level of uncertainty in the fishery, a result in agreement with previous research.

Irreversibility of investment increases the importance of inherent uncertainty in the fishery. This is particularly the case for fisheries with slow-growing resource stocks, where the occurrence of an unusually "bad" year may lead to capital lying idle for a substantial part of its economic life. However, in accordance with the work of other researchers (e.g. Lewis 1981), results obtained here show that for the linear-cost risk-neutral fishery model, optimal policies recognizing the stochastic nature of the fishery tended to perform only somewhat better than policies based on the corresponding deterministic model. In other words, the use of a deterministic model was sufficient in most cases to produce policies with near-optimal performance (on average). Indeed, it is apparent that any investment strategy sufficiently near the optimal will perform almost optimally. While this result certainly does not reduce the importance of uncertainty to fisheries management, it does imply that with linear costs and risk neutrality, economic optimization is "forgiving"; other objectives (conservation, job creation, etc.) can be pursued with little loss in the fishery's economic value, as long as the modified policy remains near the optimal strategy (with a deviation of roughly ±20% being reasonable).

Lewis (1981) has shown that this "forgiving" nature need not apply when either nonlinear costs or risk aversion are included. Since results obtained here show that investment policies can be strongly affected by uncertainty, even with linear costs and risk neutrality, the incorporation of additional nonlinearities may make the use of stochastic rather than deterministic investment policies particularly important to the fishery's performance. This will be a topic of future research.

This paper has emphasized the determination of optimal investment policies in the face of uncertainty arising through stochastic fluctuations in the resource stock. It has been assumed that the underlying population dynamics, represented by the stock-recruitment function $F(S)$, are given. However, in practice, stock-recruitment parameters (and some economic data) are known only imprecisely. In such circumstances, parameter estimates must be refined from year to year as new information becomes available. The effects of this parameter uncertainty on fisheries investment, and the role of parameter updating in overcoming these uncertainties, are considered in Charles (1983b). It is found there that initial errors in stock-recruitment parameter estimates can lead to considerable long-term overcapacity. Parameter uncertainty, it seems, plays an important role alongside stochastic variability in determining optimal investment strategies. The formulation of adaptive management policies, in which fisheries investment responds to new information, and is used in turn as a tool to acquire information, promises to be a fruitful area for further research.

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Bacteria Decomposing Amino Acids in Bulk-Stored Capelin (*Mallotus villosus*)

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KJOSBAKKEN, J., I. STORRØ, AND H. LARSEN. 1983. Bacteria decomposing amino acids in bulk-stored capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 40: 2092 – 2097.

Bacteria that decompose amino acids were isolated from anaerobically stored capelin (*Mallotus villosus*) depleted of trimethylamine oxide (TMAO). Some of the 19 isolated strains could be assigned to *Lactobacillus*, or *Bacteroidaceae*, but others were not easily identified. Bacteria of the family *Bacteroidaceae* dominated the anaerobic viable count in the capelin mass in a period after the total reduction of TMAO. Most of the isolated strains differed from each other by decomposing different sets of the free amino acids, or decomposing amino acids at different rates during anaerobic growth in capelin extracts. Most strains isolated from the fish at the later stages of storage, and notably the *Bacteroidaceae*, produced copious amounts of NH_3. Hydrophilic amino acids were the main source of the NH_3 produced. Hydrophobic amino acids and other nonprotein nitrogenous components of the capelin extract were not decomposed by any of the 19 organisms, with the exception of the quantitatively less significant hypoxanthine, which was decomposed by three of the strains. Members of the family *Bacteroidaceae* may be prominent spoilage bacteria in fish, having until now escaped attention because of their obligate anaerobic and psychrotrophic nature.

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Nous avons isolé de capelans (*Mallotus villosus*) en entreposage anaérobie et privés d'oxyde de triméthylamine (TMAO) des bactéries capables de décomposer les acides aminés. Il a été possible de classer quelques-unes des 19 lignées isolées parmi *Lactobacillus* ou *Bacteroidaceae*, mais les autres n'ont pu être facilement identifiées. Les bactéries de la famille des *Bacteroidaceae* dominent le comptage viable anaérobie dans la masse de capelans pendant un certain temps après la réduction totale du TMAO. La plupart des lignées isolées diffèrent entre elles en ceci qu'elles décomposent différents groupes d'acides aminés libres, ou qu'elles décomposent les acides aminés à des taux différents durant la phase de croissance anaérobie d'extraits de capelan. La plupart des lignées isolées du poisson après entreposage de plus longue durée, surtout les *Bacteroidaceae*, produisent de grandes quantités de NH_3. Les acides aminés hydrophiles sont la source principale de NH_3 produit. Aucun des 19 organismes ne décompose les acides aminés hydrophobes et autres composantes azotées non protéiques d'extraits de capelan, à l'exception de l'hypoxanthine, quantitativement moins importante, qui est décomposée par trois lignées. Les membres de la famille des *Bacteroidaceae* peuvent être d'importants agents de détérioration du poisson, qui ont jusqu'à maintenant échappé à notre attention à cause de leur nature anaérobie et psychrotrophe obligatoire.

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FISH such as capelin (*Mallotus villosus*), mackerel (*Scomber scombrus*), and herring (*Clupea harengus*) is the major raw material for the Norwegian fish meal industry. The fish is normally stored for a few days (unpreserved) or several weeks (preserved) in large tanks or bins (1000–5000 t) prior to processing. The storage may create processing problems. Anaerobiosis develops rapidly in bulk-stored fish, carbohydrates are oxidized and trimethylamine oxide (TMAO) is reduced to trimethylamine (TMA). The reduction of TMAO to TMA seems to be caused predominantly by facultative anaerobic, Gram negative rod bacteria which represent the primary bacterial growth during the spoilage process (Strøm and Larsen 1979).

Ammonia is produced in copious amounts in the fish mass after a lag period, and several free amino acids are degraded concomitantly (Kjosbakken et al. 1983). Ammonia most probably originated from the degradation of amino acids (free and protein bound), and represents a loss in the yield of protein in the fish meal. The isolated strains from the primary bacterial growth decompose very few amino acids and pro-

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duce minute amounts of ammonia when growing anaerobically in fish extracts (Strøm and Larsen 1979). We postulated that hitherto unidentified bacteria, delayed in their development compared to those producing TMA, were responsible for the production of ammonia always encountered in fish stored in bulk for some period.

The objective of the present study was to isolate and characterize the NH_3 producing bacteria from bulk-stored capelin, and identify the dominating source of NH_3.

Cultures and Chemical Analyses

Water extracts of capelin were used for the isolation and cultivation of bacteria. Capelin, caught in September (summer capelin) or in March (winter capelin) were frozen within a day of capture and stored at −35°C. Suitable portions were thawed and tightly packed in glass jars, stoppered to ensure anaerobic conditions. The jars were kept at 5°C until all TMAO in the fish mass had been reduced to TMA (3−5 d). The TMAO-free fish was mixed with an equal volume of tap water and homogenized with an Ultra Turrax TP 18/2. The homogenate was centrifuged at 13 000 × *g* for 1 h at 4°C, and the supernatant filtered through Whatman No. 1 filter paper to remove solidified lipids. The filtrate was heated to 95°C, cooled to 25°C and coagulated protein removed by centrifugation at 13 000 × *g* for 15 min. The supernatant was boiled for 5 min and clearified by centrifugation at 30 000 × *g* for 30 min. Sodium chloride was added to 1% (w/v).

The capelin extracts were further prepared according to Hungate (1969) for the cultivation of strictly anaerobic bacteria. In the enrichment experiments sodium thioglycolate, sodium dithionite or cysteine (0.05% w/v) were used as reducing agents, while sodium thiglycolate (0.035% w/v) was used in experiments to estimate the anaerobic viable count. Resazurin (0.0001% w/v) was used as red/ox indicator. The pH was adjusted to 7.0−7.5. Nitrogen purified as described by Hungate (1969) was used as inert gas. For liquid cultures, 5 mL extract was distributed in 15 mL Bellco Anaerobic Culture Tubes (Bellco, Vineland, NJ.). Capelin extract with 2% agar (Difco) was spread on the inner surface of the Bellco tubes using "roll tube technique." Extreme care was taken to exclude oxygen at every stage during isolation and incubation as specified by Hungate (1969). Bacterial cultures were incubated at 15±1°C. Anaerobic dilutions were done in tap water containing sodium chloride (1% w/v), yeast extract (Oxoid, 0.25% w/v) and resazurin (0.0001% w/v), pH 6.9, prepared as described by Hungate (1969).

Pure cultures were tested for Gram reaction according to Seim et al. (1971), catalase reaction and Kovacs oxidase reaction according to Harrigan and McCane (1966), and benzidine reaction according to Deibel and Evans (1960). Bergey's Manual (Buchanan and Gibbons 1974) was used for the classification of the organisms.

Free amino acids, taurine and anserine were analyzed in picric acid extracts of the cultures on a Beckman Model 120C Amino Acid Analyzer (Strøm and Larsen 1979; Storrø 1979). Cysteine + cystine were determined according to Sullivan et al. (1942). Creatine was estimated using Jaffe's reaction, TMAO, TMA and ammonia using Conway's micro diffusion technique in trichloroacetic acid extracts (Kjosbakken and Larsen 1981). Hypoxanthine was analyzed enzymatically (Jørgensen 1974).

Volatile fatty acids from acidified samples were extracted with diethyl ether as described by Dawes et al. (1971). Acetic, propionic, and butyric acid were determined quantitatively by gas−liquid chromatography using a 2-m stainless steel column packed with Neopentyl Glycol Succinate (10% w/w) and phosphoric acid (2% w/w) on Chromosorb P and FID-detector (Pedersen 1976; Storrø 1979). *n*-Valeric acid was used as internal standard.

Results

Enrichment, Enumeration, and Characterization of Bacteria

The capelin extract used as growth medium to enrich for the amino acid decomposing bacteria was purposely prepared from fish incubated anaerobically until all TMAO had been reduced to TMA. The reduction is caused by the bacteria developing early in the fish. They use carbohydrate and lactate as energy source and TMAO as H acceptor, but decompose very few amino acids. They produce little NH_3 and stop growing when the supply of TMAO is exhausted (Strøm and Larsen 1979; Strøm et al. 1979). Thus, using a TMAO exhausted fish extract as enrichment medium, the primary bacterial growth in the spoilage process will be suppressed, and the development of organisms using the residual components of the fish extract, such as amino acids, should be relatively favored.

Enrichment cultures were obtained in two ways. By the first method capelin, caught in the summer, incubated anaerobically for 11 d at 5°C and thus well in the process of spoiling, was used as inoculum in the anaerobically prepared capelin extract in Bellco culture tubes. Growth after 3 d was inoculated successively in the same medium 3 times and then spread on the surface of agar medium in roll tubes. Six strains, designated A to F, were selected for further studies.

By the other method liquid samples were taken from anaerobically incubated winter capelin mass (5°C) at suitable intervals until the day 18, diluted anaerobically, and aliquots spread directly on the surface of agar medium in roll tubes. Pure cultures were isolated and 13 strains, designated A1 to A3 and B1 to B10, were selected for further studies. A1 to A3 were isolated from the capelin mass before TMAO were completely reduced and B1 to B10 at a later stage of storage.

All organisms isolated grew quite well anaerobically in TMAO exhausted, liquid capelin extract. They were all non-spore-forming, nonmotile rods. Other characteristics are listed in Table 1. Some of the strains had the characteristics of *Lactobacillus*, others of *Bacteroidaceae*, others could not easily be assigned to a taxon on the basis of the limited tests carried out.

The number of bacteria in the capelin mass, able to grow anaerobically on TMAO exhausted capelin extract was determined. The anaerobic viable count reached a maximum of about 10^9/mL after 7 d, which coincided with the total reduction of TMAO, then gradually dropped to about 10^8/mL after 18 d (Fig. 1). The organisms isolated in pure culture were

TABLE 1. Properties of bacterial strains isolated from enrichments on trimethylamine oxide exhausted capelin extracts.

Strain[a]	Anaerobicity	Gram reaction	Catalase reaction	Kovacs oxidase reaction	Benzidine reaction	NH_3 production in capelin extracts[b]	Taxon affiliation
A1	Facultative	−	−	+	+	−	
A2	"	−	?	+	+	−	
A3	"	+	−	−	−	−	*Lactobacillus*
B1	"	?	?	−	−	+	
B2	"	+	−	+	+	−	
B3	"	+	−	+	+	−	
B4	"	−	+	−	−	+	
B5	Obligate	−	−			+	*Bacteroidaceae*
B6	"	−	−			+	*Bacteroidaceae*
B7	Facultative	+	−	−	−	−	*Lactobacillus*
B8	"	+	−	−	−	−	*Lactobacillus*
B9	"	?	−	−	−	−	
B10	"	+	+	−	−	+	
A	"	+	−	−	−	+	*Lactobacillus*
B	"	+	?	−	−	+	*Lactobacillus*
C	Obligate	−	−			+	*Bacteroidaceae*
D	Facultative	+	?	−	−	+	*Lactobacillus*
E	"	+	+	−	−	+	
F	"	+	+	−	−	+	

[a]A1−A3: isolated from colonies on agar medium inoculated with a suspension of winter caught capelin which was incubated anaerobically for 4 d at 5°C; B1−B10: the same but capelin incubated 12−18 d; A−F: isolated from liquid enrichments; inoculum, summer caught capelin, incubated anaerobically for 11 d at 5°C.

[b]− negligible, + >10 mmol/L NH_3 produced.

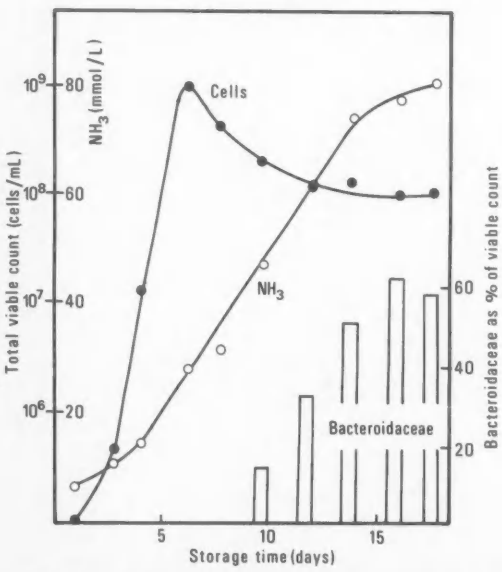

FIG. 1. Bacterial counts and NH_3 production in capelin stored anaerobically at 5°C. ●——● anaerobic viable count on trimethylamine oxide exhausted capelin extract; ○——○ ammonia; bars designate no. of *Bacteroidaceae* in % of viable count.

partly selected from colonies of different appearance (size, shape, color), partly from colonies representing bacteria present in abundance in the fish mass at different stages of the spoilage (A series vs B series, Table 1).

A particular type of colony appeared and became dominant in the roll tubes containing inocula from fish at the later stages of storage. Described according to Conn et al. (1957) the colonies were white, circular, raised, and with a slightly undulated margin. The bacteria were obligate anaerobic, Gram negative rods and could be assigned to the family *Bacteroidaceae*. They accounted for 50−60% of the anaerobic viable count in the fish at the later stages of storage (Fig. 1). Further tests have revealed the close relationship of these bacteria to the genus *Fusobacterium* (Dyrset 1979). The same type of organism was also among the dominating in liquid enrichment cultures inoculated with summer caught capelin.

ABILITY TO DECOMPOSE NITROGENOUS COMPOUNDS

The capelin extract contained the common amino acids in amounts suitable for analysis, except arginine and cysteine which were added ad libitum (Table 2). This fortified extract was used to test the ability of the 19 isolated strains of bacteria to decompose amino acids anaerobically. The cultures were incubated well into the stationary phase (6 d) before analysis. Most of the organisms produced considerable amounts of NH_3 during their growth in the capelin extract, but some produced only negligible amounts (Table 1). The organisms not producing NH_3 decomposed very few amino acids; those

TABLE 2. Amino acid decomposing ability of isolated strains when growing anaerobically at 15°C in trimethylamine oxide exhausted capelin extract. −, +, ++ designate < 10%, 10−70%, > 70% reduction, respectively, of the content of free amino acid in the medium when growth had reached the stationary phase (6 d). *w*, winter caught capelin used for strain A1−A3 and B1−B10; *s*, summer caught capelin used for strain A−F.

	Concentration in sterile medium (mM)		Ammonia production														
			Negative							Positive							
							Lactobacillus						*Lactobacillus*		*Bacteroidaceae*		
	Capelin *w*	Capelin *s*	A1 A2	B2	B3	B9	A3	B7	B8	B1	B4 B10	E F	A B	D	B5	B6	C
Aspartic acid	0.46	3.7	++	++	++	−	−	−	−	++	++	++	−	−	−	+	−
Glutamic acid	3.50	7.2	−	−	−	−	−	−	−	++	++	+	−	−	++	++	++
Lysine	0.37	3.3	−	−	−	−	−	−	−	−	−	−	−	−	++	++	+
Histidine	0.48	1.4	−	−	−	−	++	−	++	−	−	−	++	−	+	+	++
Arginine	4.33	2.0	−	−	+	−	++	++	++	++	−	−	++	−	−	+	−
Serine	0.50	2.4	++	++	++	+	+	+	−	+	++	++	−	++	+	++	++
Threonine	1.91	5.0	−	−	−	−	−	−	−	++	++	++	−	++	++	++	+
Glycine	3.92	6.2	−	−	−	−	−	−	−	−	−	−	−	−	++	++	+
Proline	1.93	4.5	−	−	−	++	−	−	++	−	−	−	−	−	++	++	++
Cysteine	3.14	3.1	−	++	−	−	−	−	+	+	++	++	+	+	++	++	++
Methionine	1.08	3.9	−	−	−	−	−	−	−	++	++	++	−	++	++	+	+
Alanine	5.90	12.1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Valine	2.98	6.9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Leucine	3.36	9.7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Isoleucine	1.80	5.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phenylalanine	1.35	3.7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tyrosine	0.16	2.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

producing NH_3 generally decomposed more amino acids (Table 2).

The three strains of *Bacteroidaceae* were most active in their attack on amino acids, decomposing 9−11 different amino acids. Most strains differed from each other by decomposing different sets of amino acids or decomposing amino acids at different rates. The 19 strains in these ways displayed 16 different patterns.

Serine was the amino acid most commonly decomposed. Lysine and glycine were attacked only by the three strains of *Bacteroidaceae*, tyrosine only slowly by one of the *Lactobacillus* strains. None of the strains attacked alanine, valine, leucine, isoleucine, and phenylalanine. Tryptophane was not tested for.

The capelin extract used as growth medium for the bacteria contained, in addition to free amino acids, the following non-protein nitrogenous compounds in considerable amounts: creatine, TMA, anserine, hypoxanthine, and taurine. None of the 19 strains of bacteria were able to decompose creatine, TMA, anserine, and taurine anaerobically. Only 3 strains decomposed hypoxanthine, and 2 of them only slowly.

The growth medium also contained some peptides, but these were apparently not decomposed with the liberation of amino acids by any of the 19 organisms, since no increase was observed in the level of any amino acid in the medium upon growth of the bacteria.

The isolated strains showed different ability to produce volatile fatty acids when growing anaerobically in capelin extracts. All NH_3 producing strains, except strain A and B, generated acetic and propionic acid. All three strains classified as *Bacteroidaceae* generated butyric acid (1.7−5.2 mmol/L) in addition to acetic acid (7−33 mmol/L) and propionic acid (1.3−2.6 mmol/L). The other NH_3 producing strains generated 8−17 mmol/L acetic acid and 2−13 mmol/L propionic acid. The strains which produce no NH_3, produced small amounts of acetic and no propionic and butyric acid.

Discussion

During bulk-storage of capelin TMAO is relatively rapidly converted to TMA; only little NH_3 is produced at this stage. Strong NH_3 production sets in only after a lag period (Kjosbakken et al. 1983). The bacteria isolated in the present work from capelin during the reduction of TMAO (6 d at 5°C, strains A1−A3) produce little or no NH_3 when growing anaerobically in capelin extract. In contrast, several of the organisms isolated from the capelin mass in the later stages of storage (12−18 d at 5°C, strains B1−B10) produced NH_3 in considerable amounts (Table 1), notably those of the family *Bacteroidaceae* which developed concomitantly with the NH_3 production and dominated the anaerobic viable count in the later stages of the storage period (Fig. 1). These observations support the contention that strong NH_3 production represents a second phase of the anaerobic spoilage process, due to the development of a secondary biota of bacteria.

The capelin extract used as growth medium for the amino acid decomposing bacteria we isolated contained, in addition to amino acids, a number of other nitrogenous compounds which might possibly serve as source of NH_3 produced by the bacteria. Kjosbakken and Larsen (1981) report that non-protein nitrogenous compounds (NPN) are present in winter-caught capelin muscle at the start of storage in the following mole proportions (relative to total NPN): TMAO 43%,

TABLE 3. Amino acids decomposed during bulk storage of capelin compared to amino acids decomposed by the isolated bacterial strains.

	Amino acids decomposed	
	in bulk stored capelin (Kjosbakken et al. 1983)	by bacteria isolated from capelin
Lysine	+	+
Histidine	+	+
Arginine	+	+
Threonine	+	+
Serine	+	+
Aspartic acid	+	+
Glutamic acid	+	+
Glycine	+	+
Proline	—[a]	+
Methionine	−	+
Valine	−	−
Alanine	−	−
Isoleucine	−	−
Leucine	−	−
Phenylalanine	−	−
Tyrosine	+	+

[a]Data inconclusive.

creatine 28%, free amino acids 10%, taurine 7%, anserine 3%, hypoxanthine 4%. Together with some NH_3 (5%) these compounds account for very near 100% of total NPN. In the capelin extract used as growth medium in the present study all TMAO was accounted for as TMA. The other NPNs were present in amounts corresponding to those found in the capelin muscle. None of the isolated bacterial strains were able to decompose TMA, creatine, taurine, and anserine anaerobically. Only 3 of the 19 strains decomposed hypoxanthine. These findings conform with those of Kjosbakken et al. (1983) that, except for a number of the common amino acids, the NPNs are remarkably resistant to degradation during bulk storage of capelin, i.e. in the presence of the mixed, natural population of the bacteria developing in the capelin mass. The findings give further support to the argument of Kjosbakken et al. (1983) that amino acids are the chief source of NH_3 in the anaerobic spoilage process. The degradation of amino acids might well affect the nutritional value of the fish and cause a loss in the yield of fish meal.

Each bacterial strain decomposed a limited number of amino acids, and the kinds decomposed differed from strain to strain (Table 2). We do not know at present to which extent these organisms utilize the amino acids in their growth process, but it is reasonable to assume that they are of considerable importance. The many different decomposition patterns observed might thus reflect that the various organisms do not all compete with each other for their growth substrate, and therefore to an extent can develop independently of each other. This might explain the varied biota of bacteria actually found in the spoiling fish.

The 19 isolated strains of bacteria decomposed the typical hydrophilic amino acids. Hydrophobic amino acids (valine, leucine, isoleucine, alanine, and phenylalanine) were not attacked (Table 2). These observations are in accordance with those reported by Kjosbakken et al. (1983) for capelin stored anaerobically for a corresponding time period under the influence of the mixed population of bacteria (Table 3). The content of the free, hydrophobic amino acids increased in the capelin mass with storage time, apparently as a result of proteolysis and inability or poor ability of the spoilage bacteria to decompose them (Kjosbakken et al. 1983). Since the 19 isolated strains, when taken together, would seem to mimic the changes taking place under anaerobic storage of the fish, we feel that the isolated strains are pertinent representatives of the natural spoilage biota. Upon prolonged anaerobic storage of fish (more than 3 wk at 5°C), also the hydrophobic amino acids were decomposed, apparently as a result of the development of still another biota of bacteria, as yet unidentified (Dyrset 1979).

Special attention should be drawn to the dominating occurrence of representatives of the family *Bacteroidaceae* in the spoiling fish. Bacteria of this type have mainly been reported from humans and warm-blooded animals. We know of only one report about their occurrence in fish; they were shown to inhabit the gastrointestinal tract of some living freshwater fishes (Trust et al. 1979). We have met them in different catches of anaerobically stored fish from cold waters, and they are enriched in anaerobically incubated fish extracts when inoculated with samples of fish in the process of spoiling. Preliminary experiments have shown the psychrotrophic nature of the isolated strains; no growth occurred at temperatures above 20°C. These organisms seem to play a particularly important role in the anaerobic decomposition of amino acids since they decompose more amino acids than other anaerobic bacteria isolated from fish, and they produce copious amounts of NH_3. In addition, the isolated strains of *Bacteroidaceae* are so far the only bacteria isolated from capelin which account for the production of butyric acid found in bulk-stored capelin after all the TMAO has been reduced to TMA (Kjosbakken et al. 1983).

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Influence of Deteriorating Water Quality on Growth and Development of Chum Salmon (*Oncorhynchus keta*) Larvae in a Japanese-Style Keeper Channel

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BAMS, R. A., AND C. N. H. LAM. 1983. Influence of deteriorating water quality on growth and development of chum salmon (*Oncorhynchus keta*) larvae in a Japanese-style keeper channel. Can. J. Fish. Aquat. Sci. 40: 2098–2104.

Chum salmon (*Oncorhynchus keta*) larvae were incubated in gravel-filled cages placed in two experimental Japanese-style keeper channels having 40 and 80 L/min total flow, at three successive locations having 0, 46 000, and 92 000 eggs (or alevins) upstream. Minimum dissolved oxygen was 6.21 mg/L, maximum un-ionized ammonia was 0.18 μg/L, pH varied from 7.18 to 6.83, and temperature ranged from 7.8 to 8.2°C. Preemergent fry showed small but significant decreases in mean fork length, with increasing distance along both channels 50 d after hatching. Weights decreased similarly but nonsignificantly in both channels. Stage of development (expressed as K_D-index) was constant in all locations except the bottom location of the low-flow channel, which showed a significant delay. Survival was uniformly high with no differential mortality among locations or flow rates. We conclude that deteriorating water quality measurably reduced larval development rate, growth rate, and yolk-conversion efficiency. Main effective factor was dissolved oxygen. Standard channel loading and flow criteria are adequate for short incubation channels only.

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Des larves de saumon keta (*Oncorhynchus keta*) ont été incubées dans des cages remplies de gravier placées dans deux chenaux de garde expérimentaux de style japonais, avec débits totaux de 40 et 80 L/min, à trois sites successifs ayant 0, 46 000 et 92 000 oeufs (ou alevins) en amont. La teneur minimale en oxygène dissous était de 6,21 mg/L, la teneur maximale en ammoniac non ionisé de 0,18 μg/L, le pH variant de 7,18 à 6,83, et la température comprise entre 7,8 et 8,2°C. Les alevins avant émergence accusèrent des diminutions de longueur à la fourche faibles mais significatives, à mesure qu'augmentait la distance le long des deux chenaux, 50 jours après éclosion. Les poids diminuèrent de la même façon, mais non significativement, dans les deux chenaux. Le stade de développement (exprimé comme indice K_D) était uniforme à tous les sites, sauf au fond du chenal à lent débit, qui montra un retard significatif. La survie a été uniformément élevée, sans qu'il y ait de différence de mortalité en fonction du site ou du débit de l'eau. Nous concluons que la détérioration de la qualité de l'eau réduit de façon notable le taux de développement, le taux de croissance et l'efficacité de conversion du vitellus des larves. Le principal facteur effectif est l'oxygène dissous. La charge standard des chenaux et les critères de débit ne sont adéquats que dans de courts chenaux d'incubation.

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DETERIORATION of water quality in rearing environments as a consequence of metabolism has long been known to affect well-being, growth, and development of hatchery reared fishes. Brockway (1950) and Burrows (1964) identified un-ionized ammonia as the main excretory product causing deleterious effects in salmonid fingerlings reared in various types of production ponds. Numerous studies have examined ammonia toxicity to juvenile and adult fish in single factor tests; two useful overviews are EIFAC (1973) and Rice and Stokes (1975). Fish reared under suboptimal hatchery conditions are not only exposed to increased ammonia levels, however, but to an interactive multifactor system also involving dissolved oxygen (DO), carbon dioxide, nitrite, and pH. Some factors acting together with ammonia have been investigated, e.g. reduced oxygen levels (Merkens and Downing 1957; Lloyd 1961), increased carbon dioxide levels (Alabaster and Herbert 1954; Lloyd and Herbert 1960), and decreasing pH (Downing and Merkens 1955; Tabata 1962).

Recently, attention has been paid to effects of waste products, specifically on eggs and alevins of salmonid fishes. Acute toxicity tests with durations of 24 h or less were reported by Penaz (1965) and Rice and Stokes (1975).

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Burkhalter and Kaya (1977) and Rice and Bailey (1980) used extended exposure times of, respectively, up to 42 and 61 d. Only Bailey et al. (1980) evaluated larval fish survival and growth as associated with various loading densities and flow rates using pink salmon (*Oncorhynchus gorbuscha*) in gravel incubators.

The purpose of the investigation was to document levels of dissolved oxygen, ammonia, pH, nitrite, and temperature experienced in four pilot-scale Japanese-style keeper channels, at known levels of cumulative fish density and water flow to help evaluate the quality of this kind of rearing environment. It further deals with responses of chum salmon (*Oncorhynchus keta*) alevins, held in cages to a stage just prior to time of emergence at various locations in these channels and exposed to the graded environmental conditions so generated, in terms of survival, growth, stage of development, and yolk-conversion efficiency.

Materials and Methods

The experiment was carried out from 1979 to 1980 at the Rosewall Creek fish-cultural facility (Department of Fisheries and Oceans, Canada), 80 km north of Nanaimo on the east coast of Vancouver Island, B.C., using gametes obtained from chum salmon of the Big Qualicum River. Two concrete Japanese-style keeper channels were used, which are located outdoors, and can be covered with rough cedar boards. For this experiment each channel was divided lengthwise by a wooden trough and across by stainless steel screens to obtain four rows of six experimental units, each unit measuring 1.4 m long by 0.8 m wide. These units were stocked at densities of 6000, 12 000, or 24 000 eggs/m^2. The flow in each subchannel was held at 40 or 80 L/min, which at a water depth of 15 cm gave nominal linear velocities of 33 and 66 cm/min. Density 12 000 and flow 40 represent the average "standard" conditions as employed both in Canada and Japan. Details of this experiment are in Bams (1982). The water supply was pumped and aerated groundwater, with a temperature of 8.1°C at time of hatching dropping to 7.3°C at emergence.

All eggs were incubated in a battery of upwelling incubators, each holding 24 000 eggs. Egg diameter was 0.76 cm and egg counts were accurate within 1%. All fish were introduced to the channel on February 12, at 428 C°-days, which was 6 d prior to 50% hatch. The channel populations originated from eggs spread evenly on Vexar screens that were the size of the experimental units and located 8 cm above the channel floor. The screens were removed when hatching was complete. The captive fish populations were contained in 15-cm-diameter by 15-cm-high sampler cages, constructed of stainless steel screen, having eight meshes to the inch (2.5 cm) and filled with gravel ranging in size from 19 to 32 mm. These cages were positioned vertically at three locations, immediately upstream, in the middle, and immediately downstream, of each row of six experimental units of each channel half. With two cages to a site, there was a total of 24 cages.

The total numbers of eggs or alevins located upstream from each of the three test sites, i.e., cage locations, were 0, 46 200, and 92 400, respectively, in each of the four subchannels. Changes in various metabolite levels along the channels were unlikely to be linearly additive due to exchange between the water medium, surface air, gravel, and concrete walls. As an arbitrary measure of ambient conditions we have used the number of preceding "egg units" (EU), where 1 EU = 6600 eggs, i.e. the base density used in the incubation experiment. The three test sites, therefore, were identified by 0, 7, and 14 EU, respectively. Arbitrary as this measure is, it is intended to convey the pragmatic nature of the stimulus used. The connotation is with a site-specific changing complex of stimuli, representative of normal field conditions, rather than the rigorously controlled laboratory experiment or the normal cultural conditions where alevins are free to roam and are likely to be exposed to different sets of conditions in time.

Water samples were collected weekly by means of a peristaltic pump connected to in situ samplers through 6.3-mm-diameter plastic tubing. The samplers were weighted, stiff plastic hoses 19.1 mm in diameter, perforated in the bottom half with numerous 1-mm-diameter holes. They were laid horizontally across the full width of each subchannel at all sampling locations and were in direct contact with the channel floor within the layer of gravel. Separate samplers of the same design were installed above the gravel in certain locations to sample the supragravel water.

Oxygen was measured by the Winkler method (precision 0.1 mg/L). Total ammonia was measured by the indophenol blue method (precision 5 μg/L total ammonia or 0.008 μg/L un-ionized ammonia at pH = 7.0, T = 8.0°C) and nitrite by the pink azo dye method (precision 0.5 μg/L). Un-ionized ammonia levels were calculated (Sigma Resource Consultants Ltd. 1979). A Corning pH meter (model 125) was used with a Fisher glass electrode and a calomel reference (precision 0.02). Details of methodology followed Stainton et al. (1977).

On April 9, at about 886 C°-days, voluntary emergence from the experimental units had begun (most units from 1 to 2%) and the sampler baskets were lifted. The fish were removed, counted, and preserved in 5% formalin. Individual fork lengths (to the nearest 0.2 mm) and total wet weights (to the nearest milligram) were determined some 6 wk after preservation. Stage of development was assessed by K_D-index (Bams 1970), expressed as

$$K_D = \frac{10\sqrt[3]{\text{weight in mg}}}{\text{length in mm}}.$$

Further details of this experiment and the results obtained in the main experimental sections are available in Bams (1982).

Results

Water Quality

Dissolved oxygen, total ammonia, nitrite, and pH were measured on four dates on intragravel samples and on two different dates on supragravel samples (Table 1). Supragravel water was always considerably less affected by cumulative fish density than intragravel water. Because the sample baskets occupied the full height of the water column they were

TABLE 1. Observed levels of dissolved oxygen, ammonia, and pH obtained at four locations along each of four subchannels, from within (4 sampling days) and above (2 sampling days) the gravel layer. Temperature of the incoming water on the four dates was 7.8, 7.9, 8.2, and 8.0°C, respectively. Dates in parentheses are those of supragravel sampling only.

Flow (L/min)	Sample location[a]			Oxygen (mg/L)				Un-ionized ammonia (μg/L)				pH			
	EU	R	L	Mar. 11(13)	Mar. 18	Mar. 25(27)	Apr. 1	Mar. 11(13)	Mar. 18	Mar. 25(27)	Apr. 1	Mar. 11(13)	Mar. 18	Mar. 25(27)	Apr. 1
Source	0	—	—	10.59	10.50	10.77	10.78	0.000	0.000	0.000	0.000	7.07	7.07	7.18	7.15
40	7	1	I	6.45	8.28	8.83	7.86	0.030	0.047	0.115	0.115	6.83	7.02	7.06	7.00
		2	I	8.57	7.35	8.66	—	0.039	0.086	0.089	0.114	7.03	7.03	7.06	7.00
		2	S	10.06	—	9.68	—	0.016	—	0.068	—	7.12	—	7.06	—
	14	1	I	8.28	7.52	7.31	7.18	0.113	0.145	0.155	0.161	6.98	6.95	7.00	6.92
		2	I	8.18	7.83	6.69	6.21	0.100	0.142	0.114	0.179	6.97	6.98	6.96	6.88
		2	S	9.24	—	8.86	—	0.064	—	0.104	—	7.06	—	7.01	—
	14/E	1	—	9.24	9.12	8.70	8.12	0.017	0.090	0.129	0.146	7.04	7.04	7.04	7.03
		2	—	9.63	8.89	8.47	8.27	0.016	0.088	0.120	0.122	7.01	7.04	7.06	7.03
80	7	1	I	9.24	9.54	9.59	9.31	0.008	0.039	0.050	0.041	7.00	7.06	7.10	7.06
		2	I	9.24	6.34	9.45	9.48	0.007	0.042	0.056	0.045	7.03	7.09	7.11	7.04
		2	S	10.01	—	10.08	—	0.000	—	0.037	—	7.12	—	7.10	—
	14	1	I	8.47	9.05	9.39	8.84	0.054	0.065	0.061	0.086	6.94	7.05	7.07	7.02
		2	I	9.05	9.05	9.06	8.74	0.020	0.065	0.083	0.075	7.01	7.04	7.07	7.04
		2	S	9.72	—	9.32	—	0.005	—	0.061	—	7.08	—	7.05	—
	14/E	1	—	10.01	9.81	9.65	9.35	0.004	0.040	0.111	0.106	7.05	7.07	7.09	7.12
		2	—	9.92	10.00	9.62	9.20	0.008	0.045	0.056	0.110	7.05	7.07	7.10	7.10

[a]1 EU (egg units) = 6600 eggs (alevins) preceding the sampling site; /E = mixed effluent water; R = subchannels 1 and 2; L = location of sampling tubes where I = intragravel and S = supragravel.

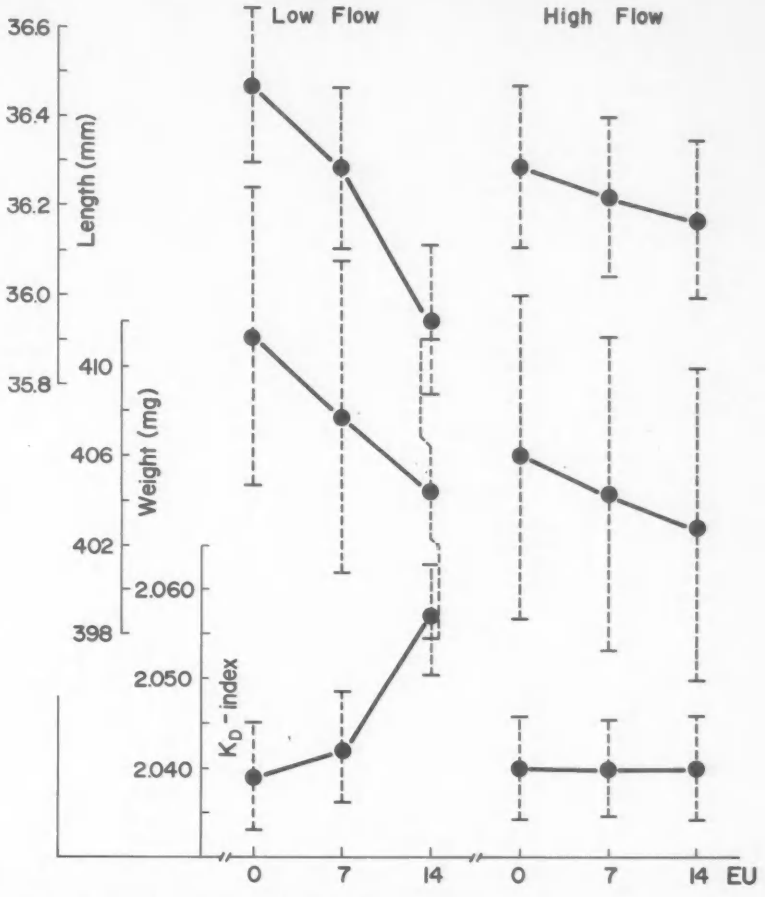

FIG. 1. Changes observed in mean fork lengths, total wet weights, and developmental index (K_D) of samples of fish incubated in cages at three successive locations in low- and high-flow keeper channels. Abscissa is EU (egg units, i.e. fish mass incubating upstream of sampler locations) where 1 EU = 6600 eggs or equivalent. Data points are means of four replicates, each containing 50 fish. Confidence ranges (95%) are given for all means.

exposed to a mix of both kinds of water, and their average metabolite levels are likely to have been intermediate between the values of the two water sampling positions. All parameters changed gradually, but not necessarily linearly, with distance downstream and changes were more substantial in low than in high flow.

The lowest DO levels measured were 6.21 mg/L (intragravel) and 8.12 mg/L (outflow), which at 8.0°C correspond to 51.3 and 67.1% saturation, respectively. These absolute levels reached Davis' (1975) protection levels *C* (56% saturation) and *B* (76% saturation) for larval salmonids. The DO of the inlet water was not at saturation, however, and to evaluate changes within the channels one must adjust to percent saturation relative to the known inlet DO. During the final 2–3 wk such adjusted intragravel DO values ranged from 57.6 to 67.9% and from 81.1 to 87.2% of inlet DO in the low and the high flow channels, respectively. Reasonable working estimates for the average DO levels inside the downstream (EU = 14) sampler baskets during this period are 60 and 75% absolute, and 72 and 82% relative to the inlet DO, in the low- and high-flow channels, respectively.

Un-ionized ammonia levels remained below 0.20 μg/L at pH = 6.88 and never reached critical levels as defined in Sigma Resource Consultants Ltd. (1979). The pH values observed ranged from 6.83 to 7.18. Nitrite concentrations, when detectable, were always below 10 μg/L.

ALEVIN CHARACTERISTICS

Mean survival was 99.65% (range 99.0−100.0%) in the high-flow and 99.33% (range 96.0−102.0%) in the low-flow channel. Only two data points were below 99% and these both occurred in the low-flow channel at a middle and a bottom end location. However, overall differences were not significant (Student t-test, $P > 0.05$).

Mean lengths of alevins decreased with increasing cumulative density in both flows (Fig. 1). Absolute differences observed were small, not exceeding 0.5 mm or about 1.5% of the grand mean, but significant ($P < 0.05$) by two-way ANOVA (F for density = 5.394, df = 2, 18, $0.025 > P > 0.01$; F for flow = 0.014, df = 1, 18, NS).

Mean weights of fish decreased similarly and consistently with a change not exceeding 2.0% of grand mean (Fig. 1) but these differences were not significant ($P > 0.05$) by two-way ANOVA (F for density = 1.350, df = 2, 18, $0.50 > P > 0.25$; F for flow = 1.927, df = 1, 18, $0.25 > P > 0.10$) due to inherently much larger variance of alevin weights.

Mean stages of development (K_D value) were the same at all three locations in the high-flow channel and the upstream location (EU = 0) in the low-flow channel (Fig. 1). However, fish at the two locations farther downstream (EU = 7 and 14) showed increasingly delayed development. Differences associated with all six locations were not significant in a two-way ANOVA (F for density = 2.401, df = 2, 18, $0.25 > P > 0.10$; F for flow = 2.307, df = 1, 18, $0.25 > P > 0.10$). However, orthogonal one-way ANOVA of the same data, for each of the three locations in the two channels, demonstrates a significant ($P < 0.01$) reduction in development rate in the low-flow channel at the downstream location:

Channel location	Mean K_D, low flow	Mean K_D, high flow	df	F	P
Upstream	2.039	2.040	1,6	0.015	>0.75
Middle	2.042	2.040	1,6	0.134	>0.50
Downstream	2.056	2.040	1,6	17.827	<0.01

Discussion

The chum alevins responded measurably to deteriorating water quality with reduced rates of growth and development. This response occurred over a limited range of DO with the minima only about 28 and 18% below available (inlet) DO in the low- and high-flow channels, respectively, during the final weeks. Furthermore, un-ionized ammonia levels exceeded 0.15 and 0.08 μg/L for the final 2 wk, but they never reached established water quality minima for long-term exposure, e.g. EIFAC (1973) at 25 μg/L and Sigma Resource Consultants Ltd. (1979) at 2 μg/L. There was no increase in mortality at these levels of metabolites, generally recognized as sublethal.

The 50-d incubation period sufficed to show the toll of accumulating metabolic effects at both flow rates: lengths decreased gradually and significantly along the length of both channels. Mean weights showed a similar change that was statistically not significant but cannot be ignored. The changes were minor in the high-flow channel (0.4% in lengths and 0.8% in weights) but more pronounced (1.5 and 2.7%) in the channel with half that flow. Reduction of growth rate in response to increasing but sublethal metabolite levels has been documented repeatedly in various salmonids (Brockway 1950; Burrows 1964; EIFAC 1973). In an 8-mo experiment with young rainbow trout (*Salmo gairdneri*) reared in two rows of seven troughs arranged in series, Larmoyeux and Piper (1973) recorded a gradual reduction in cumulative fish lengths in association with progressively changing DO and ammonia levels.

Owing to the design of this experiment, effects of unionized ammonia and reduced DO levels cannot be separated. We know that both factors were present and that either can affect developmental and growth rates to the extent demonstrated. It is clear, however, that the DO was of far greater importance to the test results than the ammonia for the following reasons. Burkhalter and Kaya (1977) found only a 3% reduction in length of rainbow trout alevins following 46 d of continuous exposure to their lowest test concentration of 50 μg/L ammonia. Rice and Bailey (1980) established a 3% reduction in length and a 7% reduction in weight in advanced pink salmon alevins exposed for 61 d to 2.4 μg/L. Their next lower concentration, 1.2 μg/L, had a small but statistically insignificant ($P > 0.05$) effect; concentrations from 0.02 to 0.4 μg/L had no visible effect. Because our highest recorded ammonia values (≤0.18 μg/L) were at least an order of magnitude below these effective levels, it is clear that ammonia alone could not have exerted sufficient influence.

It appears then that the observed reductions in development and growth rates were predominantly associated with the reduced DO levels. Such effects are poorly documented in the literature for salmonid alevins. There is an extensive literature of influence of DO on salmonid embryos up to time of hatching (Alderdice et al. 1958; Garside 1959, 1966; Silver et al. 1963) that does demonstrate significant reductions in both rates. However, the presence of the chorion greatly reduces the availability of oxygen to the embryo (Hayes et al. 1951), and the toxicity of ammonia to eggs and early alevins is very low indeed (Rice and Stokes 1975), so that these relationships cannot be applied directly to our fish, which were exposed as alevins only. Brannon (1965) reared sockeye eggs and alevins in hatchery troughs from fertilization to yolk absorption at three levels of DO (100, 50, and 25% air saturation) under constant temperature and water velocity. Fish of all three treatments hatched at 1200 temperature units (t.u.) (°F) but at significantly reduced sizes (i.e. stages of development) as DO levels diminished. We calculate daily growth rates from recorded total wet weights over a 13-d period following hatching (from 1200 to 1800 t.u.) in order of decreasing DO as 7.2, 6.5, and 5.4 mg/d, clearly demonstrating reduced growth rates at reduced DO levels. Maximum wet weights were reached in 100% DO at 39 d, in 50% DO at 41 d, and in 25% DO at more than 43 d, thus indicating significantly reduced rates of development with decreasing DO as well. Further support for reduction in rates of development and growth could be extracted from a series of reports on effects of reduced DO levels on several larval freshwater fish (Table 2). Even though the DO reductions employed in these studies exceeded those reached in ours and the period of exposure included the egg phase, it is evident that DO reductions to 50% saturation over the long term significantly influ-

TABLE 2. Times from fertilization to two life stages and lengths, with their difference, obtained at two levels of DO in different species of Lake Superior fish. Asterisk indicates DO was 70% saturation.

Source[a]	Species[b]	Temperature (°C)	90% hatch at DO[c]		First feed at DO[d]		Day[c]	Length at DO (mm)		Δ%[f]
			100	50*	100	50*		100	50*	
1	Pike 1	15	6.0	5.8	12	13	20	19.7	18.3	−7.1
	Pike 2	15	—	5.8	—	13	20	—	18.7	−5.1
2	Lake trout 1	7	81	84	101	108	131	26.5	24.8	−6.4
	Lake trout 2	10	56	58	60	76	108	26.9	23.6	−12.3
	Largemouth bass 1	20	64	64*	193	195*	20	11.6	10.7*	−8.8
	Largemouth bass 2	20	64	59	193	204	20	11.6	10.0	−13.8
	Largemouth bass 3	23	—	—*	124	124*	20	14.6	13.8*	−5.5
	Largemouth bass 4	23	—	—	124	129	20	14.6	13.4	−8.2
3	White sucker	18	7.3	7.3	12.3	12.3	22	16.7	16.4	−1.8
	Coho	7,10	63.3	66.3	93	105	119	33.8	31.6	−6.5
	Brook trout 1	8	67	66	93	95	133	24.9	24.2	−2.8
	Brook trout 2	8	—	66	—	96	133	—	24.6	−1.2
	Walleye 1	17	11	11	14	14	20	9.5	9.5	0
	Walleye 2	20	8.0	8.3	9	10	20	9.2	9.0	−2.2

[a]1 = Siefert et al. 1973; 2 = Carlson and Siefert 1974; 3 = Siefert and Spoor 1974.

[b]Pike (*Esox lucius*) 1 and 2 differed only in water exchange rate, 1 being twice that of 2. Lake trout (*Salvelinus namaycush*) 1 and 2 differed only in temperature. Largemouth bass (*Micropterus salmoides*) where 1 and 3 had DO = 70% (marked with an asterisk), 2 and 4 DO = 50%, and 1 and 2 differed in temperature from 3 and 4. Brook trout (*Salvelinus fontinalis*) where 1 had quadruple the exchange rate of 2. (White sucker, *Catostomus commersoni*; coho salmon, *Oncorhynchus kisutch*; walleye, *Stizostedion vitreum vitreum*.)

[c]Time to 90% hatching (days) except for largemouth bass, which is in hours.

[d]Time to first feeding (times as for 90% hatching).

[e]Time of sampling for lengths (days) from fertilization.

[f]Differences in lengths observed in response to lower DO.

enced rate of development and size in many fish species.

Unpublished evidence suggesting that small reductions in DO affect both length and K_D-index of salmon alevins more than low insipient ammonia levels was made available by J. O. T. Jensen (Pacific Biological Station, Nanaimo, B.C., personal communication). He worked with sockeye (*Oncorhynchus nerka*) eggs and alevins, developing from fertilization to 1080 C°-days at 10°C, in treatment combinations of DO ranging from 3.5 to 11.3 mg/L and un-ionized ammonia levels ranging from 0 to 50 μg/L. Multiple linear regression analysis indicated that DO had a much greater effect than did ammonia at the levels employed. Applying the conditions of our experiment to the model developed by Mr Jensen predicts a reduction of 3.7% in developmental rate, 3.5% of which would be owing to the 6.21 mg/L DO and 0.2% only to the 0.18 μg/L un-ionized ammonia. Similarly, the model predicts that length would be reduced by 3.8% owing to reduced DO but only an additional 0.004% due to ammonia.

This additional information, therefore, strongly suggests that the observed growth responses to deteriorating water quality were, at least to a large extent, associated with the observed reductions in DO. If ammonia contributed at all it is because its toxicity to trout and other fish is known to increase sharply with moderate reductions in DO level (Merkens and Downing 1957).

Deteriorating water quality also affected growth efficiency of the brood. Efficiency of yolk conversion in salmon alevins can be deduced from fish sizes reached at known stages of development if we can assume equal initial average yolk supplies and equal temperature regimes throughout incubation (Bams 1970). Marked differences in efficiency can thus be deduced from Fig. 1. Average stages of development were the same in all three locations of the high-flow channel, as shown by identical K_D values. Therefore, the observed decreasing lengths and weights in this channel signify decreasing efficiency of yolk conversion with increasing water quality deterioration: increasingly smaller fish were produced at the same stage of development. It can be assumed that increasingly more yolk was being diverted into metabolism associated with functions other than growth. Rice and Bailey (1980) suggested that the metabolic rate of their pink salmon alevins was increased to deal with the toxic factor, analogous to that seen in adult trout. The more pronounced size reduction observed in the low-flow channel is likely to have been influenced similarly by inefficient yolk utilization, but because there was also a pronounced delay in development (significantly higher K_D value) no direct comparison is possible. However, it seems reasonable to extrapolate the slopes shown by the other points of Fig. 1 and conclude that efficiency of yolk conversion decreased in direct proportion to increasing deterioration of water quality, most strongly so at the low flow rate.

An important interaction is shown in response to water quality at the two different flow rates (Fig. 1). Clearly, best of all conditions existed near the inlet of the *low-flow* channel: there was no delay in development and average length and weight were largest of all groups. However, with increasing distance downstream, water quality deteriorated more rapidly

at the low than at the high flow rate (Table 1) and the higher flow rate then became an asset. The smaller size of the fish held at the upstream location in the high-flow channel demonstrated that the higher flow rate extracted its own toll in energy requirements and became an advantage only after certain loading levels were exceeded.

Operationally, "standard" levels of fish density and water flow now in use for keeper channels appear adequate for short channels only. Higher than "standard" flow rates would enable lengthening the channels or increasing the density while incurring less of a penalty in terms of growth. Other operational problems are known to hinder such expansion, however, e.g. the occurrence of "clumping" (Bams 1982). Because ammonia levels remained well below critical, repeated reoxygenation of the channel water at any flow rate would allow significant extension of channel length and, hence, increase production per unit of water available.

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Stress Response and Blood Characteristics of Channel Catfish (*Ictalurus punctatus*) after Anesthesia with Etomidate

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LIMSUWAN, C., T. LIMSUWAN, J. M. GRIZZLE, AND J. A. PLUMB. 1983. Stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anesthesia with etomidate. Can. J. Fish. Aquat. Sci. 40: 2105–2112.

Continuous anesthesia of channel catfish (*Ictalurus punctatus*) with 0.6 mg/L etomidate for 96 h caused a small but statistically significant decrease in plasma protein concentration at all sampling periods. Hemoconcentration, indicated by increased total erythrocyte count, hematocrit, and hemoglobin, resulted from anesthesia with 1–4 mg/L etomidate for 30–180 min. Periodic removal of fish from 400-L troughs stressed other unanesthetized fish in the trough causing significantly increased plasma corticosteroid, glucose, and protein concentrations. Anesthetized fish were not stressed by the periodic sampling. Fish anesthetized with 3 mg/L etomidate and then confined in a net for 10 min had reduced plasma cortisol response and no significant plasma glucose increase compared with unanesthetized controls. Anesthesia did not prevent hyperchloremia that developed 3 h after the 10-min confinement. No histological changes were found in fish anesthetized with etomidate. Anesthesia with etomidate before netting could be useful when handling fish because of the reduced stress response.

LIMSUWAN, C., T. LIMSUWAN, J. M. GRIZZLE, AND J. A. PLUMB. 1983. Stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anesthesia with etomidate. Can. J. Fish. Aquat. Sci. 40: 2105–2112.

L'anesthésie continue de barbues de rivière (*Ictalurus punctatus*) à 0,6 mg/L d'étomidate pendant 96 h a causé une diminution faible mais statistiquement significative de la concentration de protéines plasmatiques à toutes les phases de l'échantillonnage. L'anesthésie à 1–4 mg/L de la même substance pendant 30–180 min a causé une hémoconcentration, indiquée par l'augmentation du nombre total d'érythrocytes, de l'hématocrite et de l'hémoglobine. L'enlèvement périodique de poissons de bassins de 400 L produisit un stress chez les autres sujets non anesthésiés dans le même bassin, avec augmentation concomittante notable des corticostéroïdes, du glucose et des concentrations de protéines plasmatiques. L'échantillonnage périodique n'a pas affecté les poissons anesthésiés. Les poissons anesthésiés à 3 mg/L d'étomidate et ensuite maintenus dans un filet pendant 10 min virent leur cortisol plasmatique diminuer, sans augmentation significative du glucose plasmatique, comparativement aux témoins non anesthésiés. L'anesthésie n'a pas empêché l'hyperchlorémie de se développer 3 h après l'emprisonnement de 10 min. Aucun changement histologique n'a été observé chez les poissons anesthésiés à l'étomidate. Dans la manutention des poissons, il pourrait être utile de les anesthésier à l'étomidate avant de les capturer au filet, le stress se trouvant ainsi atténué.

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ETOMIDATE, *R*-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate, is a nonbarbiturate, short-acting hypnotic agent (Godefroi et al. 1965) that has high potency when administered intravenously to mice, rats, guinea pigs, rabbits, and dogs (Janssen et al. 1975). Extensive clinical studies in humans indicate that etomidate produces rapid induction of anesthesia without release of histamine (Doenicke et al. 1973) and causes minimal effects on cardiovascular and respiratory functions (Morgan et al. 1975; Gooding and Corssen 1977). Hematological and histological studies did not reveal any drug-related adverse effects after daily injection of etomidate for 3 wk in rats and 2 wk in dogs (Janssen et al. 1975). Amend et al. (1982), Plumb et al. (1983), and Limsuwan et al. (1983) demonstrated that etomidate is an effective and safe anesthetic for fish.

Our objectives were to determine if fish anesthetized with etomidate undergo hematological and histological changes and if the stress response is affected. Concentrations of

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etomidate and length of time in anesthesia were selected to provide information for short-term as well as long-term anesthesia.

Materials and Methods

Histological and Hematological Changes during 4-d Exposure

Channel catfish (*Ictalarus punctatus*) fingerlings, averaging 10 g, from the Alabama Agricultural Experiment Station were acclimated to fiberglass troughs in the laboratory for 3 wk. Dechlorinated municipal water flowed through the troughs at 0.9 L/min with aeration and at a temperature of 17–19°C. The fish were fed each day during the acclimation period. Water characteristics (American Public Health Association 1975) were as follows: total alkalinity, 17–31 mg/L as $CaCO_3$; total hardness, 42–49 mg/L as $CaCO_3$; pH 7.8–7.9.

Following laboratory acclimatization, fish were transferred to 40-L aquaria containing 30 L of dechlorinated tap water 24 h before etomidate exposures. Fish were not fed while in aquaria. The water was aerated during the experiment and the temperature was 22 ± 1°C. In a static test, etomidate solution in 55% propylene glycol (Tavolek Co., Redmond, WA) was added to eight aquaria to obtain an initial nominal concentration of 0.6 mg/L, and fish in another eight aquaria served as controls. An etomidate concentration of 0.6 mg/L was chosen because it was the maximum sublethal concentration during earlier 96-h toxicity tests. Twenty test fish and 20 control fish were sampled at 24, 48, 72, and 96 h. Blood from one-half of the sampled fish was used to determine hematocrit, hemoglobin concentration, erythrocyte and leukocyte counts, and differential leukocyte counts. Blood from the other 10 test fish was centrifuged, and plasma was separated and stored at −15°C until analyzed for plasma glucose and plasma protein. Gills, trunk kidney, liver, and spleen were removed from one-half of the sampled fish for histological examination.

Blood samples were taken by severing the caudal peduncle and collecting blood in heparinized capillary tubes. Hematocrit was determined by the microcentrifugation method of Larsen and Snieszko (1961). Hemoglobin concentration was determined by the cyanmethemoglobin method of Oser (1965) using a correction factor for channel catfish as recommended by Larsen (1964). Blood for erythrocyte and leukocyte counts was transferred from a heparinized capillary tube to a blood cell diluting pipette, diluted 1:100 with Yokoyama's fluid (Yokoyama 1960), and transferred to a hemocytometer, where both erythrocytes and leukocytes were counted. Morphologic indices for erythrocytes were calculated according to Weinberg et al. (1972). Mean corpuscular volume (MCV) was calculated by dividing the hematocrit (percentage) by the erythrocyte count (millions per cubic millilitre) and multiplying by 10. Mean corpuscular hemoglobin (MCH) was calculated by dividing the hemoglobin (grams per 100 mL of blood) by the erythrocyte counts (millions per cubic millilitre) and multiplying by 10. Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing the hemoglobin (grams per 100 mL of blood) by the hematocrit (percentage) and multiplying by 100.

Differential leukocyte counts were made by examining blood smears at 1000× magnification. Blood from individual fish was smeared on a glass slide, fixed in absolute methanol, and stained with Wright–Giemsa solution (Humason 1972). Differentiation of blood cell type followed that of Grizzle and Rogers (1976).

Plasma glucose was determined by the method of Hyvarinen and Nikkila (1962). Plasma protein was determined by use of the Biuret reaction as described by Wedemeyer and Yasutake (1977).

Tissues were fixed in Bouin's solution, dehydrated in alcohol, cleared in xylene, and then infiltrated and embedded in Paraplast. The embedded tissues were sectioned at 6 μm on a rotary microtome, mounted on glass microscope slides, and stained with hematoxylin and eosin (Humason 1972).

Hematalogical Changes during Recovery from 30-min Exposure

Channel catfish were acclimated and stocked in 20 aquaria under the same conditions as described in the first experiment. The average weight of the fish used in this experiment was 10 g. Etomidate was added to 10 aquaria at 2 mg/L, and the fish in the other aquaria served as controls. Thirty minutes after anesthesia, 20 fish from two aquaria were sampled. Twenty control fish were also sampled. The remaining fish were transferred by dip net to two troughs supplied with flowing, dechlorinated water. Fish were fed daily and sampled at 24, 48, 72, and 96 h after they were transferred to freshwater. Hematological and histological methods were the same as for the previous experiment.

Hematological Changes during 24-h Exposure

Channel catfish averaging 104 g were placed into four 400-L fiberglass, rectangular troughs, 30 fish per trough, and supplied with dechlorinated tap water. Fish were fed daily during a 2-wk acclimation period. Water characteristics were as follows: total alkalinity, 29–31 mg/L as $CaCO_3$; total hardness, 40–51 mg/L as $CaCO_3$; pH 8.0–8.2; temperature 24°C.

Immediately prior to the addition of etomidate, water flow into the troughs was interrupted and aeration was supplied by air stones. Etomidate was added to three troughs for initial nominal concentrations of 1, 2, and 4 mg/L, and the fish in the fourth trough were controls. Blood samples were taken from six fish in each trough at 15, 45, 90, and 180 min and 24 h after etomidate was added.

Approximately 2.5 mL of blood from vessels in the caudal peduncle was collected from each fish in heparinized vacutainers. The caudal peduncle was then severed and four heparinized capillary tubes were filled with blood from the caudal vessels. Blood samples from vacutainers were immediately centrifuged for 15 min, and plasma was stored at −15°C for later determination of plasma corticosteriod, plasma glucose, total plasma protein, and plasma chloride. Blood collected in heparinized capillary tubes was used to determine hemoglobin and hematocrit. Plasma corticosteriod concentration was determined in 100-μL samples with Fagerlund's (1970) modification of Murphy's (1967) procedure using chicken serum

TABLE 1. Mean values (±SD) of hematological characteristics for channel catfish exposed for different durations to 0.6 mg/L etomidate as compared with unexposed control fish. Mean values that differ significantly ($P < 0.05$) from the control at each time period are indicated by an asterisk.

	24 h		48 h		72 h		96 h	
Parameter	Control	Etomidate	Control	Etomidate	Control	Etomidate	Control	Etomidate
Plasma glucose (mg/100 mL)	36.2±1.86	52.5±4.79*	31.8±2.40	26.4±1.23	40.9±3.61	31.1±4.15	31.1±3.31	27.9±2.50
Plasma protein (g/100 mL)	2.98±0.05	2.74±0.04*	2.95±0.05	2.66±0.05*	3.14±0.04	2.72±0.07*	2.97±0.07	2.48±0.03*

as the transcortin source. A standard curve, developed by plotting the reciprocal of the percent bound of corticosteroid standards between 0.5 and 10 ng, was used to determine concentration of corticosteroid. Plasma chloride concentration was determined by the method of Schales and Schales (1941). Hemoglobin, hematocrit, plasma glucose, and plasma protein were determined by the same methods used in the first experiment.

Hematological Changes after Confinement

Channel catfish averaging 395 g were placed into two 400-L fiberglass, rectangular troughs. Fish were fed daily and held for 2 wk before the experiment. Water and temperature were the same as described for the third experiment.

Prior to the addition of etomidate, water flow into the troughs was interrupted, but aeration was continued so that the dissolved oxygen concentration remained near saturation. Etomidate was added to one trough to obtain a concentration of 3 mg/L. Fifteen minutes after addition of etomidate, six fish were sampled from the group that was not anesthetized to establish a prestress level. The remaining fish (about 30 fish) from each trough were confined for 10 min in a dip net suspended in the trough. Then the fish were transferred to 112-L fiberglass troughs supplied with flowing freshwater with six fish per trough. Blood samples were taken from fish at 0.5, 3, 7, 24, and 48 h after they were transferred. All blood samples were analyzed as in the third experiment.

Statistical Analysis

Student's *t*-test was used to determine differences between control and treatment in the first experiment. Analysis of variance and Duncan's multiple range test (Steel and Torrie 1960) were used to identify differences among sample means in experiments 2, 3, and 4. All comparisons were made at the $\alpha = 0.05$ level.

Results

Histological and Hematological Changes during 4-d Exposure

During the 96-h exposure to 0.6 mg/L etomidate, channel catfish reached stage 3, plane 1 (complete loss of equilibrium but rolling movements persist) within approximately 3 min. Stages of anesthesia defined by Schoettger and Julin (1967) were used to describe anesthesia of channel catfish with etomidate. After 12 h, channel catfish showed signs of recovery toward sedation and after 24 h, all fish were in sedation. Channel catfish fingerlings showed signs of initial stimulation, evidenced by rapid swimming, during the first 5–10 s after exposure to etomidate. Failure to respond to visual stimulation occurred at 45 s and the first signs of partial loss of equilibrium was observed at 130 s. None of the fish exposed to 0.6 mg/L etomidate or controls died during this experiment.

Plasma glucose and plasma protein concentrations were the only hematological variables affected by 0.6 mg/L etomidate (Table 1). Plasma glucose levels of the anesthetized fish were significantly higher than those of unanesthetized controls after 24 h. However, plasma glucose levels for anesthetized fish returned to control level by 48 h. Plasma protein of the anesthetized fish was significantly lower than control values during the 96-h experimental period.

Erythrocyte and leukocyte morphology under 1000× magnification appeared normal. No fragmentation of cells and no abnormality in cell size and shape were observed. There were no significant differences in hematocrit, blood cell counts, differential leukocyte counts, or erythrocyte morphologic indices.

No histological changes related to etomidate were noted in the liver, gills, trunk kidney, and spleen of channel catfish exposed to 0.6 mg/L etomidate for 96 h.

Hematological Changes during Recovery from 30-min Exposure

During the 30-min exposure to 2 mg/L etomidate, channel catfish reached stage 3, plane 2 (complete loss of equilibrium and rolling movements stop) within approximately 6 min. None of the fish died during the exposure or during the 96-h recovery period. All fish recovered to normal activity approximately 35 min after they were transferred to freshwater.

Hematocrit, hemoglobin, and total erythrocyte counts increased significantly after 30 min of anesthesia (Table 2). Twenty-four hours after fish were transferred to freshwater, both anesthetized and unanesthetized groups had lower hematocrit values than the 30-min controls. Hematocrit values for both control and treated fish then returned to the initial level after 48 h. MCH and MCHC for anesthetized fish were significantly lower than for controls after 30 min of anesthesia (Table 2). These values increased to the control levels after a 24-h recovery period.

There were no significant differences in total or differential leukocyte counts, plasma glucose, and plasma protein between control and anesthetized fish in this study.

TABLE 2. Mean values (±SD) of hematological characteristics for channel catfish exposed to 2 mg/L etomidate for 30 min and during a 96-h recovery period as compared with untreated control fish. Mean values that differ significantly ($P < 0.05$) from the control at each time period are indicated by an asterisk. Mean values that differ significantly from the 30-min control are indicated by two asterisks.

Parameter	30 min		24-h recovery		48-h recovery		72-h recovery		96-h recovery	
	Control	Etomidate	Control	Etomidate	Control	Etomidate	Control	Etomidate	Control	Etomidate
Hematocrit (%)	35.9±1.90	44.5±2.40*	30.3±1.66**	29.4±0.88**	31.2±2.12	31.9±1.16	36.7±0.86	35.5±1.50	37.2±1.41	35.7±1.73
Hemoglobin (g/100 mL)	7.9±0.18	8.4±0.11*	7.4±0.20	7.3±0.35	7.4±0.18	7.6±0.19	7.9±0.33	7.8±0.19	8.0±0.22	8.1±0.27
Erythrocytes (10^6/mm^3)	2.25±0.06	2.56±0.01*	2.08±0.06	1.99±0.18	2.09±0.14	2.20±0.06	2.26±0.15	2.33±0.04	2.31±0.15	2.27±0.09
MCH (pg)[a]	35.1±0.47	32.7±0.52*	36.0±0.80	37.0±0.84	35.4±0.97	34.2±0.26	35.3±1.77	33.9±0.42	35.1±1.48	35.6±0.85
MCHC (%)[b]	22.0±0.71	18.5±0.64*	24.0±0.79	24.2±0.86	23.5±0.60	23.0±0.41	21.4±0.59	21.7±0.68	21.8±0.24	22.6±0.62

[a]Mean corpuscular hemoglobin.
[b]Mean corpuscular hemoglobin concentration.

No histological changes were observed in the liver, gills, trunk kidney, and spleen of channel catfish exposed to 2 mg/L etomidate for 30 min and during 96 h of recovery.

HEMATOLOGICAL CHANGES DURING 24-h EXPOSURE

During the 24-h exposure to 1, 2, and 4 mg/L etomidate, all fish in 2 and 4 mg/L etomidate died before the 24-h sampling period because of etomidate-induced medullary collapse. All fish in 1 mg/L etomidate lost equilibrium (stage 3, plane 1) in 2 min, and after 20 min, one-half of the fish were more deeply anesthetized (stage 3, plane 2). Anesthetized fish exhibited darker color than controls during the experimental period. Fish in 2 mg/L etomidate lost equilibrium (stage 3, plane 2) within 6 min and were darker than controls. Fish in 4 mg/L etomidate were immobilized (stage 3, plane 2) within 2 min, exhibited no respiration at 180 min, and were paler than controls. All fish in 4 mg/L etomidate were dead at the 7-h sampling period. The control fish sampled at 15 min were considered as baseline or prestressed values because controls sampled later during the experiment had been excited by the removal of fish from the troughs.

The unanesthetized group consistently had the highest corticosteroid concentrations (Table 3). Channel catfish in 4 mg/L etomidate had the next highest corticosteroid concentration at all sampling periods; however, these values were similar to the prestressed level. Fish in 1 and 2 mg/L etomidate had consistently lower plasma corticosteroid concentrations than other groups throughout the experiment. Plasma corticosteroid concentrations were significantly lower than the prestressed level at 45 and 180 min in the 1 mg/L etomidate group and at 45, 90, and 180 min in the 2 mg/L etomidate group.

Plasma glucose concentrations in unanesthetized fish increased significantly after 45 min (Table 3). Hyperglycemia remained at 180 min in unanesthetized fish, but by the 24-h sampling period the plasma glucose level had dropped to the prestressed level. Fish in 4 mg/L etomidate had the next highest concentrations of plasma glucose, but these values were not significantly higher than the prestressed level. Fish in 2 mg/L etomidate had lower plasma glucose levels than other groups. Hypoglycemia remained for 180 min in the 2 mg/L etomidate group. Fish in 1 mg/L etomidate had hypoglycemia at the 45-min and 24-h sampling periods.

Plasma protein levels from all treatments were not significantly different from the prestressed value at the 15-, 45-, and 90-min sampling periods. After 180 min and 24 h, control fish had significantly higher plasma protein levels than the prestressed level, but levels in the anesthetized fish were not significantly elevated (Table 3).

Plasma chloride concentrations were not significantly different between anesthetized and control fish at any sampling period (Table 3). All plasma chloride values were similar to the prestressed value.

Fish in 4 mg/L etomidate had the highest hematocrit and hemoglobin values at each sampling period (Table 3). Fish in 1 and 2 mg/L etomidate had the next highest values, and the control group consistently had the lowest hematocrit and hemoglobin. There were significant differences in hematocrit values between the controls and fish in 4 and 1 mg/L

TABLE 3. Mean values (±SD) of hematological characteristics for channel catfish exposed continuously to different levels of etomidate. Means followed by the same letter within a column are not significantly different ($P < 0.05$). Mean values that differ ($P < 0.05$) from the 15-min control are indicated by an asterisk.

Duration and treatment	Corticosteroid (ng/mL)	Glucose (mg/100 mL)	Protein (g/100 mL)	Hematocrit (%)	Hemoglobin (g/100 mL)
15 min					
Control	19.6±3.64 a	49.8±5.20 a	3.27±0.06 a	35.0±1.95 a	8.7±0.39 a
Etomidate 1 mg/L	12.9±1.17 a	33.7±4.17 b	3.17±0.10 a	39.0±2.34 a	8.8±1.03 a
Etomidate 2 mg/L	16.4±2.39 a	31.1±3.07 b	3.06±0.12 a	39.6±0.91 a	9.5±0.53 a
Etomidate 4 mg/L	17.4±1.74 a	51.4±8.96 a	3.26±0.06 a	41.1±2.31 a	10.1±0.59 a
45 min					
Control	80.3±25.50* a	118.3±10.56* a	3.30±0.27 a	35.2±1.47 c	8.8±0.33 b
Etomidate 1 mg/L	8.2±1.45* b	23.0±1.34* b	2.99±0.67 a	39.8±0.97 b	9.5±0.52 a b
Etomidate 2 mg/L	11.2±0.65* b	28.1±2.94* b	3.14±0.13 a	40.9±1.80 a b	10.1±0.69 a b
Etomidate 4 mg/L	18.1±0.90 c	41.3±13.45 b	3.02±0.10 a	45.8±1.47* a	10.7±0.62* a
90 min					
Control	53.7±7.08* a	72.6±9.69 a	3.27±0.09 a	35.3±1.81 c	9.1±0.34 a
Etomidate 1 mg/L	13.7±8.41 b c	40.8±3.33 b	2.71±0.29 a	41.9±1.96* a b	9.9±0.26 a
Etomidate 2 mg/L	8.2±1.28* c	26.1±1.21* c	3.22±0.18 a	39.5±0.87 c b	9.1±0.28 a
Etomidate 4 mg/L	21.3±1.99 b	58.8±8.14 a b	2.97±0.11 a	45.6±1.60* a	10.2±0.32* a
180 min					
Control	69.5±5.28* a	79.8±3.74* a	3.86±0.13* a	36.0±1.50 b	9.2±0.25 a
Etomidate 1 mg/L	5.8±0.80* c	45.0±6.42 c b	3.53±0.16 a	42.4±1.38* a	9.5±0.75 a
Etomidate 2 mg/L	7.8±0.71* c	30.7±2.73* c	2.87±0.20 b	40.2±1.62 a b	8.9±0.49 a
Etomidate 4 mg/L	28.9±4.74 b	62.3±10.76 a b	2.96±0.18 b	44.8±2.63* a	10.0±0.32* a
24 h					
Control	38.7±6.87* a	45.6±3.73 a	3.77±0.16* a	35.1±1.48 a	8.3±0.33 a
Etomidate 1 mg/L	16.0±1.83 b	27.3±2.07* b	3.12±0.12 b	39.0±1.99 a	8.7±0.36 a

etomidate for 45, 90, and 180 min. Hemoglobin concentrations of controls and fish in 4 mg/L etomidate were significantly different after 45 min. Fish in 1 mg/L etomidate had a significant increase in hematocrit value over control after 45 min of anesthesia. When compared with prestressed values, fish in 4 mg/L etomidate had significant increases in hematocrit and hemoglobin values at 45-, 90-, and 180-min sampling periods. Fish in 1 mg/L etomidate had a significant increase in hematocrit values at 90- and 180-min sampling periods.

HEMATOLOGICAL CHANGES AFTER CONFINEMENT

No fish died during the confinement and 48-h recovery. The 3 mg/L etomidate induced loss of equilibrium (stage 3, plane 2) within 3 min.

Plasma corticosteroid concentrations in both anesthetized and unanesthetized fish were higher than the prestressed level (Table 4). Plasma corticosteroid concentrations in unanesthetized fish peaked at 30 min during the recovery and were higher than levels in the anesthetized groups through the first 7 h of recovery. Plasma corticosteroid concentrations from anesthetized fish increased after confinement and remained at about the same level for all sample periods. Corticosteroid levels of anesthetized fish at 3 and 7 h were significantly higher than prestressed level; at other sample times the variation within the samples was too high for the treatment means to be significantly different.

Anesthetized fish had a small increase in plasma glucose levels after netting and confinement but plasma glucose levels were not significantly different from the prestressed level. Unanesthetized fish had higher plasma glucose levels than anesthetized fish in all samples during the first 7 h of recovery. After 24 h there was no significant difference between anesthetized and unanesthetized fish nor between stressed and prestressed groups.

Plasma chloride levels in anesthetized and control fish increased significantly from the prestressed level after 3 h of recovery and remained high until 7 h (Table 4). After 24 h of recovery, however, the plasma chloride levels from both groups had dropped to near the prestressed level. Plasma chloride concentrations from anesthetized and unanesthetized fish increased again, but not significantly, at the 48-h sampling period.

Plasma protein, hematocrit, and hemoglobin values from anesthetized and unanesthetized fish did not vary significantly from the prestressed level throughout the experiment.

Discussion

The lowest concentration of etomidate tested (0.6 mg/L) caused hyperglycemia and a reduced plasma protein concentration, but these changes did not occur in response to higher concentrations of etomidate. The hyperglycemia may reflect increased physical activity because there is an excitability in channel catfish exposed to etomidate. Hyperglycemia following increased activity in fish was reported by Nakano and Tomlinson (1967). Hyperglycemia did not occur in fish anesthetized for 48–96 h with 0.6 mg/L etomidate or in any of the fish exposed to higher etomidate concentrations and not

TABLE 4. Hematological comparison (mean ±SD) of anesthetized (3 mg/L etomidate) and unanesthetized channel catfish after netting and confinement for 10 min. Mean values within a column that are significantly different from control are indicated by an asterisk. Mean values that are significantly different from prestressed values are indicated by two asterisks.

Recovery period and treatment	Corticosteroid (ng/mL)	Glucose (mg/100 mL)	Chloride (mmol/L)
Prestressed			
Control	19.8±4.41	44.3±8.79	113±4.50
30 min			
Control	87.3±10.76**	130.0±10.54**	123±4.02
Etomidate	39.8±11.81*	56.6±4.93*	110±7.96
3 h			
Control	69.1±4.23**	103.3±8.42**	133±4.60**
Etomidate	42.8±6.7*, **	39.5±5.80*	131±2.87**
7 h			
Control	62.1±4.23**	60.1±6.87	138±7.14**
Etomidate	37.2±4.60*, **	31.2±1.93*	137±6.74**
24 h			
Control	46.4±12.97	52.1±10.24	116±3.93
Etomidate	44.3±12.23	51.8±4.22	116±4.07
48 h			
Control	37.5±6.64	49.9±5.47	127±5.53
Etomidate	38.7±7.50	44.6±2.72	131±7.18

stressed by other factors. The reason for the low plasma protein concentration of the anesthetized fish in 0.6 mg/L etomidate is not known.

Channel catfish anesthetized with 2 mg/L etomidate for 30 min had hemoconcentration indicated by increased hemoglobin and erythrocyte concentrations and hematocrit. Hemoconcentration in fish anesthetized with MS-222 was reported by Houston et al. (1971a, 1971b), Reinitz and Rix (1977), Soivio et al. (1977), and Smit et al. (1979). Splenic contraction may have caused the hemoconcentration by adding erythrocytes to the circulation. The hematological characteristics indicating hemoconcentration had returned to control values 24 h after recovery from anesthesia.

A decrease in hematocrit compared with 30-min controls occurred 24 h after recovery from anesthesia. This change was in both etomidate-exposed and control fish and probably indicates a stress reaction to the test conditions (Hattingh and van Pletzen 1974; Hattingh et al. 1975).

The reduced MCH and MCHC in channel catfish anesthetized with 2 mg/L etomidate for 30 min may be caused by a lower hemoglobin concentration in new erythrocytes released into the peripheral circulation. Both the MCH and MCHC had returned to control values 24 h after recovery from anesthesia. A similar decrease in MCHC was observed in rainbow trout anesthetized with neutralized MS-222 or benzocaine (Soivio et al. 1977).

The concentrations of etomidate used in this study were not harmful to fish, as indicated by lack of histological changes and only minor hematological effects. Lack of histological changes is consistent with the results of experiments with four other fish species exposed to etomidate (Amend et al. 1982).

Increased blood concentrations of corticosteroids and catecholamines occur in fish that are stressed, and these hormonal changes are correlated with the establishment of hyperglycemia (Nakano and Tomlinson 1967; Mazeaud et al. 1977; Miles et al. 1974; Strange et al. 1977, 1978; Strange and Schreck 1978; Barton et al. 1980; Strange 1980; Tomasso et al. 1980). Increases in plasma protein concentration of fish are also associated with stress (Bouck 1972). Netting and confinement for 10 min initiated a stress response manifested as increased plasma corticosteroid levels in both anesthetized and unanesthetized fish and as hyperglycemia in unanesthetized fish. Plasma corticosteroid, glucose, and protein concentrations in fish receiving no etomidate increased when they were frightened or tried to avoid capture while other fish were periodically sampled from the same trough. Increased plasma corticosteroid levels in fish after removal of other fish from a trough were reported by Redgate (1974).

Etomidate reduced the stress response resulting from either confinement or periodic sampling of channel catfish. Plasma corticosteroid concentration of unanesthetized fish 30 min after confinement was more than double the concentration in anesthetized fish. The changes in hematocrit, hemoglobin concentration, and plasma corticosteroid, glucose, and protein concentrations after periodic sampling were also less in fish anesthetized with etomidate than in unanesthetized fish. Davis et al. (1982) found that etomidate reduced the stress response of striped bass (*Morone saxatilis*).

Hyperchloremia occurred in both anesthetized and unanesthetized fish after confinement for 10 min, but plasma chloride levels were not affected by etomidate exposure alone or by periodic sampling. Hyperchloremia of confined channel catfish in our study was probably caused by hypoxia or some other factor rather than by a stress response because hypochloremia is usually associated with the stress response of freshwater fish (Mazeaud et al. 1977; Hunn 1969;

Wedemeyer 1972; Umminger and Gist 1973; Tomasso et al. 1980). This conclusion is also supported by the lack of change in chloride levels of fish stressed by the periodic removal of other fish and having elevated plasma corticosteroid and glucose values.

The lack of serious side effects in fish exposed to etomidate indicates that this highly effective anesthetic should receive further testing for use as a fish anesthetic. The stress-reducing capabilities of both anesthetic and sedative concentrations of etomidate could make this drug useful in fish culture to reduce the stressful effects of handling.

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Simulation and Application of Stock Composition Estimators[1]

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COOK, R. C. 1983. Simulation and application of stock composition estimators. Can. J. Fish. Aquat. Sci. 40: 2113–2118.

Stock composition estimates for mixed populations may be obtained with a classification scheme and the results of classifying individuals from this mixed population and from the component subpopulations. Several estimators have been proposed. Simulation studies were conducted over a wide range of practical possibilities. These studies showed that the simplest estimator, together with a constraint, was more than adequate for practical application. Implementation of the constraint provides for a dramatic reduction in effort for scientists involved in stock identification work. Statistically valid, conservative confidence intervals were obtained with existing variance formulae.

COOK, R. C. 1983. Simulation and application of stock composition estimators. Can. J. Fish. Aquat. Sci. 40: 2113–2118.

On peut estimer la composition de stocks dans des populations mixtes à l'aide d'un schéma de classification et des résultats du classement d'individus de cette population mixte et des sous-populations qui la composent. Plusieurs estimateurs ont été proposés. Des simulations ont été effectuées sur une vaste gamme de possibilités pratiques. Ces études ont démontré que l'estimateur le plus simple, joint à une contrainte, est approprié à une application pratique. Cette contrainte diminue de façon dramatique l'effort des scientifiques impliqués dans l'identification des stocks. Les formules de variance actuelles donnent des intervalles de confiance conservateurs, statistiquement valides.

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MANY fishery investigations are directed at determining the stock composition of populations in areas of intermingling. Estimates of the racial components of these populations are useful for management and scientific applications. By utilizing a classification scheme, such estimates are possible. However, the investigator cannot always make direct comparison between the classification results and the composition of the population unless the classification scheme is error free. To make nearly unbiased estimates of the proportion of each class present in the population, one must correct for classification error rates. Thus, these estimates are a function of the classification results and of the classification scheme.

Several estimators have been provided. The classification matrix correction procedure (Cook and Lord 1978) gives a simple estimator. Since it is a nonlinear estimator, it is biased. To correct for this bias, Pella and Robertson (1979) have recommended one of two improved estimators. Regardless, values of less than zero or greater than one may be obtained for the proportion of a stock present in the mixed population. To avoid this possibility, a constrained estimator has been developed. The implications of applying this constraint are assessed with an extensive application involving 164 estimates. A series of simulation studies producing 1500 estimates are then used to compare all of the estimators. The experimental situations cover a wide range of practical possibilities to determine guidelines for application.

Estimators

Let the mixed population consisting of unknown proportions from each of M subpopulations be represented by the vector $\boldsymbol{U}$, where the element U_j is the actual proportion of the population belonging to subpopulation j. The problem is to estimate $\boldsymbol{U}$. Assume that there exists some classification scheme that will assign individuals to each of M classes corresponding to the M subpopulations. Further, assume that the probabilities of assigning an individual to classes i will vary depending on which subpopulation j the individual truly belongs to. Thus, the classification scheme may be represented by the matrix $\boldsymbol{C}$, where the element C_{ij} is the probability that an individual from subpopulation j would classify as class i ($\sum_i C_{ij} = 1, \forall_j$). If the results of classifying the entire population are denoted by the vector $\boldsymbol{R}$, where the element R_i is the proportion of the population that would classify as class i, then the following relation exists:

$$\boldsymbol{CU} = \boldsymbol{R}, \text{ or } \boldsymbol{U} = \boldsymbol{C}^{-1}\boldsymbol{R}.$$

To estimate $\boldsymbol{U}$, $\boldsymbol{C}$ and $\boldsymbol{R}$ must be estimated. To estimate $\boldsymbol{C}$, test

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samples are removed from each of the subpopulations. Thus, the subpopulations must be segregated either before or after mixing.

The individuals comprising the test sample from each subpopulation j are classified with the scheme. The size of the sample from subpopulation j is denoted by n_j, and the number of individuals from the sample that classified as class i is denoted by d_{ij}. Thus, $\hat{C}_{ij} = d_{ij}/n_j$. Now, the mixed population is sampled to obtain $\hat{\boldsymbol{R}}$. The results of classifying the individuals in the unknown sample are tallied, letting s_i be the number of individuals that classified as class i. An estimate of $\boldsymbol{R}$ is now possible: $\hat{R}_i = s_i/n_u$. An estimate for $\boldsymbol{U}$ is evident:

$$\text{(1a)} \quad \hat{\boldsymbol{U}} = \hat{\boldsymbol{C}}^{-1}\hat{\boldsymbol{R}}.$$

This estimator will reduce to the formulation of Worlund and Fredin (1962) for the case of two stocks (Cook and Lord 1978). Restated:

$$\text{(1b)} \quad \hat{U}_i = \frac{\hat{R}_i - \hat{C}_{ij}}{\hat{C}_{ii} - \hat{C}_{ij}}.$$

Pella and Robertson (1979) have provided two statistical bias corrections for the inverse of a random variable by adapting Fuller's[3] general results to this problem. Restated:

$$\text{(2)} \quad \bar{\boldsymbol{U}} = (\hat{\boldsymbol{C}} + \hat{\boldsymbol{G}})^{-1}\hat{\boldsymbol{R}}$$

$$\text{(3)} \quad \ddot{\boldsymbol{U}} = (\boldsymbol{I} - \hat{\boldsymbol{C}}^{-1}\hat{\boldsymbol{G}})\hat{\boldsymbol{C}}^{-1}\hat{\boldsymbol{R}}.$$

The (i,k)th element of $\hat{\boldsymbol{G}}$ is given by

$$\text{(4)} \quad \hat{G}(i,k) \simeq \left(\hat{C}^{ki}\hat{C}_{ik}(1 - \hat{C}_{ik}) - \sum_{\ell \neq k} \hat{C}^{k\ell}\hat{C}_{ik}\hat{C}_{\ell k}\right)/n_k$$

where $\hat{C}^{ki}$ is the (k,i)th element of $\hat{\boldsymbol{C}}^{-1}$.

The initial solution obtained with equations (1), (2), or (3) may contain unrealistic values; some $\hat{U}_i$ may be negative or some may be greater than one. Thus, the initial solution region is given as the hyperplane defined by the relation $\Sigma\hat{U}_i = 1$. By imposing the side condition that $0 \leq \hat{U}_i \leq 1$, the region becomes a portion of the hyperplane, as bounded by M linear equations of dimension $M - 1$. The equations defining the constrained solution region may be obtained by simply replacing the A_i, one at a time for each equation, with the value zero in the relation $\Sigma A_i\hat{U}_i \leq 1$, and setting the remaining A_i to one. When a nonfeasible solution is obtained, the best correction would be to choose as the constrained estimate that point in the constrained region at minimum distance from this nonfeasible solution. This constrained estimate would be on the boundary. The problem becomes simply that of minimizing the distance to the closest boundary equation. The method of Lagrange or analytic geometry gives

$$\bar{U}_i = A_i\hat{U}_i - A_i\frac{\left(\sum_i A_i\hat{U}_i\right) - 1}{\sum_i A_i} \quad \text{and}$$

$$\text{(5)} \quad A_i = f(\hat{U}_i) = 1, \ \hat{U}_i > 0$$
$$= 0, \ \hat{U}_i \leq 0.$$

This correction is valid where multiple negative entries are obtained for $\hat{U}_i$. In such cases the distance being minimized is from the nonfeasible estimate to a constrained estimate at the intersection of equations defining the boundary of the constrained region. The number of such intersecting equations corresponds to the number of negative values in the nonfeasible estimate.

Another possibility exists. The correction may result in negative estimates. In such cases, the correction is reapplied to the new nonfeasible estimate. This situation will occur when the point nearest the initial nonfeasible estimate is on a boundary equation outside the constrained region. It is possible to redefine the $f(\hat{U}_i)$ so that this situation does not occur; however, the correction itself provides for this, and it is much simpler to reapply the correction than to incorporate it into $f(\hat{U}_i)$.

Imposition of this constraint is not absolutely necessary; however, it can save a considerable amount of effort. There is an inherent assumption that the unknown mixed population consists only of individuals from the subpopulation that are modeled with the classification scheme. Thus, the investigator must model all those subpopulations that have a reasonable chance of occurring in the mixed population. It is most probable that negative mixing proportion estimates will occur for some of these subpopulations, particularly when they are not present or are in very low abundance. The problem is compounded when the individuals sampled from the unknown mixed population are cross-tabulated to provide several estimates. It is intuitively satisfying to simply drop those classes with negative estimates and repeat the analysis: the original decision selecting the number of subpopulations to include in the analysis was conservative, and the revised decision includes fewer subpopulations. In practice, this may become tedious, as it was for the following application. With more than three subpopulations and a number of applications, the task may become arduous. With only three subpopulations, 1 three-class and up to 3 two-class analyses might be needed for each application. This is tolerable. However, with four subpopulations 1 four-class, up to 4 three-class, and up to 6 two-class analyses could be needed. With five subpopulations up to 1 five-class, 5 four-class, 10 three-class, and 10 two-class analyses could occur. The situation rapidly becomes difficult to manage, particularly for studies of wide scope. Clearly, an alternative would be desirable. The above correction provides this alternative.

A disadvantage of the traditional approach is that when a subpopulation present in a very small proportion receives a negative estimate it is dropped and no interval estimate is obtained. When the constraint is applied the estimate will be corrected to equal 0 and a confidence interval may be esti-

[3]W. A. Fuller. 1970. Mimeographed class notes, Statistics 638, winter 1969–70. Iowa State Univ. Stat. Lab. 56 p.

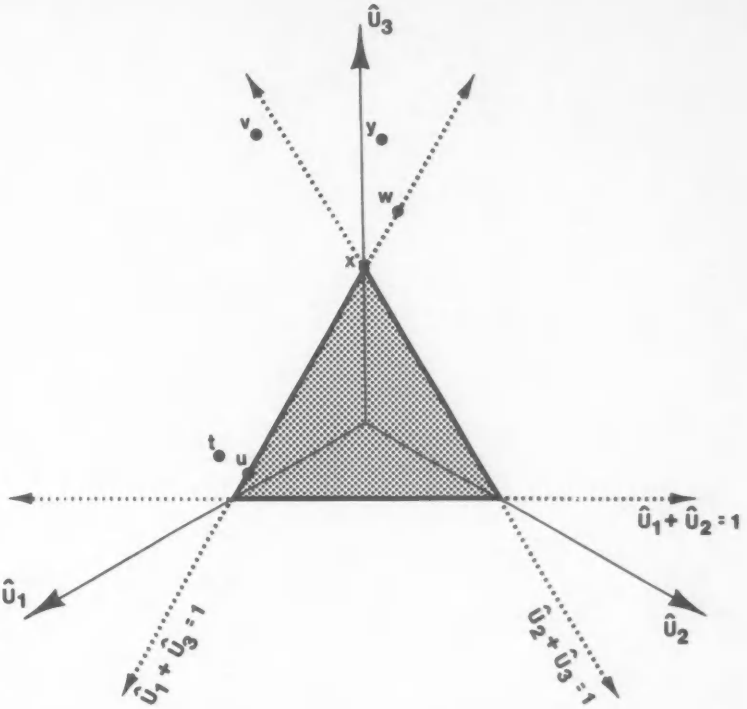

FIG. 1. Constrained region (shaded area) in the $\hat{U}_1 + \hat{U}_2 + \hat{U}_3 - 1 = 0$ plane and some examples of three-class estimates.

mated with existing methods. This is desirable for managers and scientists interested in monitoring the minor subpopulations within the composite population.

Application and Examples

In the following application (Cook 1982a), the composition of the sockeye (*Oncorhynchus nerka*) population in and around the Japanese landbased driftnet fishery was of interest. It was desirable to determine whether these sockeye had originated on the Kamchatka Peninsula, in Bristol Bay, or in the Gulf of Alaska. Five years and four age classes were investigated so that 19 separate analyses were conducted (sample sizes were inadequate for one of the years). A nonparametric scale pattern recognition technique was utilized as the classification scheme (Cook 1982b). When negative mixing proportion estimates were obtained, the appropriate two-class analyses were conducted. Thus, it was necessary to conduct 32 additional applications of the pattern recognition technique. By using the correction, this could have been avoided: the analyses could have been conducted in a fraction of the time. It is of interest to determine whether the conclusions of the study might have been altered by using the constrained estimator.

With the samples taken from mixed population, 164 estimates were obtained for various statistical areas and time periods. The general conclusions were that sockeye salmon from the Kamchatka Peninsula were found throughout the study area, that fish from Bristol Bay were detectable in the northeastern portion of the area studied, and that Gulf of Alaska salmon were generally absent or in very low undetectable levels of abundance. Negative mixing proportion estimates were obtained for 113 of the 164 original estimates because of the true mixing proportion of close to or equal to zero for the Bristol Bay and Gulf of Alaska subpopulations and the associated variability of the estimator. Constrained estimates were obtained for these 113 situations. The differences between the estimates obtained with reapplication of the pattern recognition and with the correction averaged 0.023173 for the Bristol Bay estimates, −0.0038607 for the Gulf of Alaska estimates, and −0.0193126 for the Kamchatka Peninsula estimates. Overall, there were few differences and the conclusions of the study would not be altered. For most of these estimates the differences were small; however, in some isolated circumstances the differences were quite large. These cases were associated with either small sample sizes or poor ability of the pattern recognition scheme to discriminate. These factors are associated with the variability of the

TABLE 1. Population values and observed results for three-class simulations.

Simulation No.	No. of experiments	Population values C	Population values U	Sample sizes Test	Sample sizes Unknown	Observed results: $\hat{U}$ MSE	Observed results: $\hat{U}$ % CI incl.
1	50	0.9000 0.0500 0.0500 0.0500 0.9000 0.0500 0.0500 0.0500 0.9000	0.3333 0.3333 0.3333	100 100 100	100	0.003384 0.003436 0.002471	98 96 100
2	50	0.6000 0.0500 0.2000 0.3000 0.9000 0.1000 0.1000 0.0500 0.7000	0.3333 0.3333 0.3333	100 100 100	100	0.009478 0.006224 0.007296	98 98 96
3	50	0.6000 0.0500 0.3000 0.3000 0.8000 0.0500 0.1000 0.1500 0.6500	0.3333 0.3333 0.3333	100 100 100	100	0.020112 0.007723 0.011196	94 98 96
4	50	0.9000 0.0500 0.0500 0.0500 0.9000 0.0500 0.0500 0.0500 0.9000	0.2000 0.3000 0.5000	100 100 100	100	0.002510 0.003021 0.003427	96 96 100
5	50	0.8000 0.0500 0.2000 0.1000 0.9000 0.1000 0.1000 0.0500 0.7000	0.2000 0.3000 0.5000	100 100 100	100	0.006217 0.003916 0.006805	96 98 96
6	50	0.9000 0.0500 0.0500 0.0500 0.8000 0.1000 0.0500 0.1500 0.8500	0.2000 0.3000 0.5000	100 100 100	100	0.002784 0.005286 0.006207	96 96 92
7	50	0.6000 0.0500 0.2000 0.3000 0.9000 0.1000 0.1000 0.0500 0.7000	0.2000 0.3000 0.5000	100 100 100	100	0.007744 0.006583 0.004412	100 92 100
8	50	0.6000 0.0500 0.3000 0.3000 0.8000 0.0500 0.1000 0.1500 0.6500	0.2000 0.3000 0.5000	100 100 100	100 100	0.018497 0.007639 0.014232	98 96 94
9	50	0.9000 0.0500 0.0500 0.0500 0.9000 0.0500 0.0500 0.0500 0.9000	0.1000 0.2000 0.7000	100 100 100	100	0.001677 0.002130 0.003240	100 98 100
10	50	0.6000 0.0500 0.2000 0.3000 0.9000 0.1000 0.1000 0.0500 0.7000	0.1000 0.2000 0.7000	100 100 100	100	0.006677 0.005242 0.007337	100 96 98
11	50	0.6000 0.0500 0.3000 0.3000 0.8000 0.0500 0.1000 0.1500 0.6500	0.1000 0.2000 0.7000	100 100 100	100	0.010339 0.005047 0.007721	98 100 98

estimator (Cook and Lord 1978), and since there is no way to determine which of two estimates is "best" in such situations, the lesson is that variability should be well controlled. If the variability is well controlled, either the constraint may be imposed or the classification procedure repeated when negative mixing proportion estimates are obtained. For this example, the effort associated with the constrained estimator is several orders of magnitude less than that associated with reapplication of the pattern recognition scheme.

To illustrate the correction, a figure and some examples are in order. Figure 1 shows the $\hat{U}_1 + \hat{U}_2 + \hat{U}_3 - 1 = 0$ plane as viewed perpendicularly from above. The constrained region is shaded and the three equations defining the boundaries are given by broken lines. These lines divide the nonfeasible area into six regions in which $f(\hat{U}_i)$ operates uniquely on the elements of $\hat{U}$.

One estimate provided by this study was $\hat{U}' = [0.167\ -0.099\ 0.932]$ for the three subpopulations, respectively. By imposing the constraint, the estimate becomes $\bar{U}' = [0.117, 0.000\ 0.883]$. In Fig. 1, $\hat{U}$ and $\bar{U}$ are shown by points t and u, respectively. Differences of this magnitude are commonplace in studies where the variability of $\hat{U}$ is well controlled. In most cases, the initial estimates should be within the constrained region. For applications where one of the subpopulations occurs at a very low percentage (or not at all) in the mixed population, the constraint must be applied often.

When the variability of $\hat{U}$ is not well controlled or when a large number of $\hat{U}$ are obtained (say, from independent samples in time and space), then a few rather large changes will

TABLE 2. Population values and observed results for two-class simulations.

Simulation No.	No. of experiments	Population values		Sample sizes		Observed results: $\hat{U}$	
		C	U	Test	Unknown	MSE	% CI incl.
1	50	$\begin{pmatrix}0.9500 & 0.0500\\0.0500 & 0.9500\end{pmatrix}$	$\begin{pmatrix}0.5000\\0.5000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	50	0.006803	90
2	100	$\begin{pmatrix}0.9000 & 0.1000\\0.1000 & 0.9000\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.003125	94
3	50	$\begin{pmatrix}0.8500 & 0.1500\\0.1500 & 0.8500\end{pmatrix}$	$\begin{pmatrix}0.9000\\0.1000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	50	0.007150	96
4	50	$\begin{pmatrix}0.8000 & 0.2000\\0.2000 & 0.8000\end{pmatrix}$	$\begin{pmatrix}0.5000\\0.5000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.011228	86
5	50	$\begin{pmatrix}0.7500 & 0.2500\\0.2500 & 0.7500\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	50	0.017447	100
6	100	$\begin{pmatrix}0.7000 & 0.3000\\0.3000 & 0.7000\end{pmatrix}$	$\begin{pmatrix}0.9000\\0.1000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.013297	95
7	50	$\begin{pmatrix}0.9000 & 0.2000\\0.1000 & 0.8000\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.004503	96
8	100	$\begin{pmatrix}0.8000 & 0.3000\\0.2000 & 0.7000\end{pmatrix}$	$\begin{pmatrix}0.9000\\0.1000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.007829	95
9	100	$\begin{pmatrix}0.7000 & 0.4000\\0.3000 & 0.6000\end{pmatrix}$	$\begin{pmatrix}0.5000\\0.5000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	100	0.044188	93
10	50	$\begin{pmatrix}0.9000 & 0.1000\\0.1000 & 0.9000\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	50	0.006602	96
11	50	$\begin{pmatrix}0.9000 & 0.1000\\0.1000 & 0.9000\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	25	0.011433	94
12	50	$\begin{pmatrix}0.9000 & 0.1000\\0.1000 & 0.9000\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}50\\50\end{pmatrix}$	25	0.014457	90
13	50	$\begin{pmatrix}0.7500 & 0.2500\\0.2500 & 0.7500\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	25	0.032850	94
14	50	$\begin{pmatrix}0.7500 & 0.2500\\0.2500 & 0.7500\end{pmatrix}$	$\begin{pmatrix}0.7500\\0.2500\end{pmatrix}$	$\begin{pmatrix}50\\50\end{pmatrix}$	25	0.041539	94
15	50	$\begin{pmatrix}0.8000 & 0.3000\\0.2000 & 0.7000\end{pmatrix}$	$\begin{pmatrix}0.9000\\0.1000\end{pmatrix}$	$\begin{pmatrix}100\\100\end{pmatrix}$	25	0.012846	100

be realized by applying the constraint. Two such examples have been chosen from the estimates in this application strictly for the purposes of illustration. Resolution was not satisfactory for typical examples. In the first of these examples, $\hat{U}' = [0.096\ -0.533\ 1.437]$, the first imposition of the constraint gave $\bar{U}' = [-0.170\ 0.000\ 1.170]$, and the second imposition of the constraint gave $\bar{\bar{U}}' = [0\ 0\ 1]$. In Fig. 1 these estimates are illustrated by points v, w, and x, respectively. This example corresponds to situations in which the initial correction results in negative estimates, as discussed previously. For the next example, $\hat{U}' = [-0.137\ -0.249\ 1.386]$ and $\bar{\bar{U}}' = [0\ 0\ 1]$. Points y and x correspond to these estimates and illustrate the change for multiple negative entries in $\hat{U}$.

Simulation Studies

Simulation studies were conducted to examine the performance of $\hat{U}$, $\bar{U}$, and $\ddot{U}$ under varying circumstances.[4] The constraint was imposed when needed. For each situation, population values for C and U were defined. The sample sizes and the number of experiments conducted for each situation were also defined. Using these values and the formulae

[4]Details are available from the author. Complete set of tabular data is available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

presented earlier, sampling procedures were simulated and estimates obtained. For each situation, these repeated experiments allowed calculation of the observed mean squared error for point estimators and percent confidence interval inclusion using the variance estimator of Pella and Robertson (1979). Appraisals of the various formulae were then possible.

Table 1 presents the user-specified inputs and observed results for those three-class situations studied; Table 2 does so for the two-class situations in which $\hat{U}_1 = 1 - \hat{U}_2$. Therefore, the mean squared error and percent confidence interval inclusion are identical for both elements of $\hat{U}$, and only one value is presented in Table 2. There were no practical differences between $\hat{U}$, $\tilde{U}$, and $\ddot{U}$; therefore, only the results for $\hat{U}$ are listed.

The additional complexities involved in $\tilde{U}$ and $\ddot{U}$ are unnecessary. This is in agreement with the assertion of Box (1971) that the magnitude of the bias is small in relation to the standard error for nonlinear estimators. Pella and Robertson (1979) also agree that biases appear negligible in comparison with magnitudes of variance of the estimators; however, they prefer $\tilde{U}$ based on limited numerical studies that reveal a slight advantage when sample sizes are small and classification rules are poor. They also note that "differences among variances of the three estimators become negligible either as rules improve or sample sizes become large." The slight advantage perceived by Pella and Robertson (1979) exists only when classification accuracies and sample sizes are so small that the variabilities of the estimates are too large for practical application: confidence intervals may range from 0 to 1. The simulations contained in this paper reveal that for practical confidence intervals the performances of the three estimators are nearly equivalent. The constrained $\hat{U}$ provided statistically valid, conservative confidence statements. For the three-class case, simultaneous confidence intervals were calculated using chi square approximation. On an element-by-element basis one would expect the confidence statements to be conservative, since the defined probability level (90% in this paper) is for all three population values to be included simultaneously by their respective intervals. However, this does not explain the degree to which the confidence intervals are conservative. For example, in simulation 1 there were at most three cases in which simultaneous confidence interval inclusion was not attained. (If the violation by the first element occurred in the same experiment as one of the violations by the second element then there would be two cases in which simultaneous confidence interval inclusion was not achieved.) Thus, the minimal simultaneous inclusion level would be 94%. This conservative nature is also revealed in Table 2 because for the two-class case, simultaneous confidence interval inclusion and individual element confidence interval inclusions are identical. (The normal approximation was used for the two-class situation.) Overall, it is apparent that confidence interval estimates are conservative over a wide range of potential applications.

Discussion and Conclusions

Equation (1) will provide nearly unbiased estimates of the mixing proportion of component stocks in a mixed stock population. Corrections for bias are not needed in practical application because the magnitude of this bias is very small over the range of practical possibilities. When negative values are obtained for the mixing proportion, the constraint defined by equation (5) may be imposed and the effort required with the traditional reapplication(s) of the classification procedure may be avoided. The resulting estimate may be used with Pella and Robertson's (1979) variance formulae to obtain statistically valid, conservative confidence intervals. Because these confidence intervals are conservative, 90% intervals are recommended.

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Acute Effects of Toxaphene and Its Sediment-Degraded Products on Estuarine Fish[1]

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The acute effect of toxaphene and the dechlorinated products that are formed in reducing sediments were determined for two estuarine fish, spot (*Leiostomus xanthurus*) and white mullet (*Mugil curema*). The reductively dechlorinated mixture was prepared by incubating toxaphene with anoxic salt marsh sediment for 3 wk and isolating the products, which were analyzed by packed and glass capillary gas chromatography. The 96-h LC50 (flow through) for toxaphene was 0.92 and 2.88 μg/L for spot and mullet, respectively, agreeing well with reported toxicities for the two species. Sediment-degraded toxaphene was almost as toxic to spot (LC50 = 1.10 μg/L) as the parent pesticide, while mullet were about three times more susceptible to the degraded form (LC50 = 1.02 μg/L). Bioaccumulation was similar for both forms of toxaphene, with factors of 2000–6000 over the 96-h exposure period.

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Les effets aigus du toxaphène et des produits déchlorés formés dans des sédiments réducteurs ont été déterminés sur deux poissons estuariens, le spot (*Leiostomus xanthurus*) et le muge blanc (*Mugil curema*). Le mélange déchloré a été préparé en incubant, pendant trois semaines, le toxaphène avec des sédiments de marais salés anoxiques et en isolant les produits, qui ont ensuite été analysés par chromatographie en phase gazeuse sur colonne capillaire remplie et tube capillaire en verre. La CL50 après 96 h (à débit continu) du toxaphène est de 0,92 et de 2,88 μg/L pour le spot et le muge blanc respectivement, ce qui s'accorde bien avec les toxicités mentionnées chez ces deux espèces. Le toxaphène dégradé par les sédiments est presque aussi toxique pour le spot (CL50 = 1,10 μg/L) que le pesticide parent, alors que le muge blanc est environ trois fois plus susceptible à la forme dégradée (CL50 = 1,02 μg/L). La bioaccumulation est semblable pour les deux formes de toxaphène, avec facteurs de 2 000–6 000 durant la période d'exposition de 96 h.

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TOXAPHENE, a complex mixture of over 180 chlorinated bornanes, is a widespread contaminant in the aquatic environment. Residues have been identified in fish from such diverse areas as the South and North Atlantic, Alpine lakes, and the Antarctic Ocean (Zell and Ballschmiter 1980), Sweden and the Baltic Sea (Jansson et al. 1979), and the Great Lakes (Ribick et al. 1982). In remote areas, toxaphene contamination of fish can sometimes exceed tissue concentrations of polychlorinated biphenyls (PCB) (Zell and Ballschmiter 1980). Toxaphene is acutely toxic to aquatic organisms at low microgram per litre concentrations, with bioconcentration factors for fish and oysters of 10^3–10^4 (Schimmel et al. 1977). Chronic toxicity tests have shown that low nanogram per litre (parts per trillion) levels produce growth inhibition and bone composition changes in three species of freshwater fish (Mehrle and Mayer 1975a, 1975b, 1977).

Concentrations of toxaphene that have deleterious chronic effects on fish are being approached or exceeded in the environment. Toxaphene concentrations in rainfall along the South Carolina coast during 1976–78 ranged from 10 to 490 ng/L, with a yearly average of 45 ng/L (Harder et al. 1980), and averaged 160 ng/L in the Chesapeake Bay region during the summer (Munson 1976). We have also measured toxaphene in South Carolina lakes and rivers in the range of 3–40 ng/L. By comparison, Mehrle and Mayer (1975a, 1975b, 1977) found that 39–60 ng/L toxaphene increased bone calcification in young fish.

The composition of toxaphene changes markedly after only a few days of contact with anoxic estuarine sediments (Williams and Bidleman 1978). This transformation appeared to be caused by chemical rather than biological processes and is likely a reductive dechlorination such as that observed in metabolic systems (Khalifa et al. 1976; Saleh and Casida

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1978). Some of the toxic components of toxaphene have been isolated, and one, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (toxicant B) (Khalifa et al. 1974), has been reductively dechlorinated in a variety of chemical and biological systems (Saleh and Casida 1978). Two dechlorinated products of toxicant B vary greatly in their degree of toxicity to fish, being approximately 2 and 30 times less toxic than the parent compound.

Fewer than 6% of the total number of toxaphene components have been isolated and individually examined for toxicity, but a recent study suggests that a considerable number of them are acutely toxic to fish. Isensee et al. (1979) separated toxaphene into nine fractions on a silica gel column and determined the 96-h LC50 of each fraction to bluegills (*Lepomis macrochirus*). Only the first two and last two column fractions showed reduced toxicity compared with the unfractionated toxaphene, while the middle five fractions gave LC50s equal to or lower than those of the original pesticide.

Limited data exist on the toxicity changes produced by degradation of the entire toxaphene mixture. During the late 1960s several Wisconsin lakes were poisoned with toxaphene to kill rough fish. Hughes and Lee (1973) isolated toxaphene from lake sediments 10 mo after treatment and found, as did Williams and Bidleman (1978), that the GC fingerprint was shifted in favor of early eluting compounds. Subsequently, Lee et al. (1977) reported reduced bluegill mortality for toxaphene extracted from lake sediments compared with the parent pesticide. Since toxaphene undergoes very rapid composition changes in anoxic estuarine sediments, we investigated differences in toxicity for parent and sediment–degraded toxaphene to two species of estuarine fish, spot (*Leiostomus xanthurus*) and white mullet (*Mugil curema*).

Experimental

Preparation of Degraded Toxaphene

Approximately 250–400 mg of toxaphene (lot number X-19098-8-78, 68.5% Cl, obtained from Hercules, Inc., Wilmington, DE) was dissolved in a small volume of ethanol and added to 1 kg of sediment. The sediment was obtained from the 2–10 cm layer in North Inlet Estuary, a high-salinity marsh near Georgetown, SC. Analysis of unspiked sediment revealed less than 5 ng/g dry wt of PCB, DDT residues, or toxaphene. The sediment–toxaphene mixture was thoroughly stirred, sealed in a glass jar, and kept at room temperature for 20 d.

After the degradation period, the sediment was slurried with reagent-grade acetone, and the solvent was separated from the mud by decantation and filtration. The acetone was reduced to about 100 mL by flash evaporation, 300 mL of distilled water was added, and the degraded toxaphene was partitioned into petroleum ether. The petroleum ether extract was concentrated and cleaned up by alumina column chromatography. Elemental sulfur was removed by treatment with metallic mercury (Goerlitz and Law 1974) or by the tetrabutylammonium sulfite procedure of Jensen et al. (1977). Final cleanup was achieved by shaking the extract with several volumes of concentrated sulfuric acid. Toxaphene, degraded toxaphene, and many other chlorinated hydrocarbons are unaffected by acid treatment, but most oxygen- and nitrogen-containing compounds are removed (Kawano et al. 1969; Veierov and Aharonson 1978). The cleaned up extract was diluted to 100 mL with petroleum ether. This stock solution, approximately 2 mg/mL, was further diluted with acetone for bioassays.

The concentration of degraded toxaphene in the stock solution was determined by packed column GC (180 cm long × 0.2 cm inside diameter, glass) using electron capture (ECD) or flame ionization detection (FID). EC–GC work was carried out on a 3% OV-225 column using a Varian 3700 instrument, while FID–GC analyses were done on a 3% Dexsil-300 column mounted in a Packard 7300 series chromatograph. Quantification was achieved by comparing the total peak area (excluding the solvent front) of degraded toxaphene chromatograms with those of parent toxaphene. Areas were determined using a Varian CDS-111 integrator–data system.

Bioassays

All bioassays were conducted at the University of South Carolina's Belle W. Baruch Marine Laboratory located at North Inlet Estuary. The air-conditioned wet laboratory was equipped with flowing unfiltered seawater (normally 32–35‰ salinity), which was delivered to an 80-L tank placed above the bioassay system to serve as a constant pressure head. Water temperatures were ambient; however, a heater was placed in the 80-L tank to ensure that the water temperature did not fall below 18°C. A siphon fed water from the constant head tank into a glass delivery tube, which emptied into glass mixing and splitting chambers. The splitting chamber divided the effluent into two 40-L glass aquaria. The bioassay system consisted of six such setups, allowing experiments at six test concentrations to be performed in duplicate.

Toxicant was added in acetone carrier above the mixing chambers through inert tubing connected to six motor-driven syringes. The rate of toxicant addition was 1.0 mL/h. Seawater was delivered at 25 L/h, for a turnover rate in the test aquaria of 8 volumes/d. Toxicant was removed from the effluent by percolation through a bed of charcoal and activated charcoal.

Fish were collected from North Inlet Estuary by dipnet and were held in flowing seawater until use. Except for the first experiment, fish were acclimated to laboratory conditions for 5 d prior to toxicity tests. Fish were fed frozen brine shrimp (*Artemia*, Living World brand) during the holding period and were not fed during the 96-h experiments. Mullet were collected during January–February 1980 and again during February–March 1981. Test individuals weighed 110–210 mg and ranged from 25 to 28 mm standard length. Spot were collected during February–March 1981. The weight and standard length of test individuals were 130–240 mg and 26–32 mm, respectively.

Toxicity tests were carried out by placing 10–20 individuals in each test chamber and exposing them to parent or degraded toxaphene for 96 h. Concentrations of toxicant were monitored by extracting 0.5–2.0 L of water with petroleum ether and analyzing the extract by EC–GC. Measured concentrations averaged about 20% below desired levels and

varied no more than 10% during a 96-h bioassay experiment. Average monitored concentrations were used to derive toxicity values. Two types of control experiments were carried out. In the first, reagent-grade acetone was delivered into the aquaria throughout the 96-h run. In the second, an extract of unspiked estuarine mud was used to determine whether substances extracted from the sediment itself had a deleterious effect on the fish. The mud extract was subjected to the same cleanup procedures as those used in the degraded toxaphene preparation. The final unspiked mud extract was tested at concentrations equivalent to three times those present in the degraded toxaphene experiments. LC50 and confidence intervals were derived using the graphical log−probit method of Litchfield and Wilcoxon (1949).

Analysis of Body Burdens

Whole fish (3−10 individuals) were ground with anhydrous sodium sulfate and soxhlet extracted with petroleum ether. The extract was concentrated, cleaned up by alumina column chromatography and concentrated sulfuric acid, and analyzed by packed column EC−GC. Bioconcentration factors (BCF) were calculated from the residues in the fish (on a wet wt basis) and the monitored toxicant concentration during the 96-h experiment.

Results and Discussion

Characterization of Degraded Toxaphene

The 20-d incubation period in the formation of degraded toxaphene was chosen, as much of the rapid alteration of toxaphene occurs within the 1st mo (Williams and Bidleman 1978). Two batches of degraded toxaphene were prepared within a 2-mo period and examined by EC−GC. Chromatograms of the two preparations were very similar and showed definite alterations from the normal toxaphene GC pattern (Fig. 1). A third batch was prepared a year later and, after confirming that it was similar to the first two, the three preparations were combined to provide a representative degraded toxaphene product for further GC characterization by glass capillary GC (Fig. 2) and bioassays.

Establishing the concentration of degraded toxaphene in the bioassay stock solution is a difficult task, since the reductively dechlorinated product, like parent toxaphene, is a highly complex mixture. By comparing the total area of degraded toxaphene chromatograms with those of the parent pesticide, we were able to estimate the degraded toxaphene concentration using FID−GC and EC−GC; the apparent concentration determined by FID−GC is about 20% higher.

FID response is proportional to the moles of carbon passing through the detector. We tested the ability of the FID to give an accurate response to the carbon in a chlorinated hydrocarbon by injecting 4−17 μg of parent toxaphene and comparing the total integrated pattern area with a response factor derived from n-C_{14}−n-C_{24} hydrocarbons. The toxaphene used contained 68.5% Cl, which corresponds closely to an octachlorobornane in average composition ($C_{10}H_{10}Cl_8$: 68.6% Cl, 29.0% C, 2.4% H). Assuming 29% for the carbon content of toxaphene, we recovered an average of 101% ± 15% of the total carbon injected (19 experiments). The FID thus responds accurately to the carbon skeleton of toxaphene and presumably also to the carbon in degraded toxaphene. Eleven analyses of degraded toxaphene calculated from the response factor for parent toxaphene gave 2593 ± 430 μg/mL. The same result was obtained by dividing the carbon content of the solution (752 μg/mL, quantified using n-alkane response factors) by 0.29, the percent carbon in parent toxaphene.

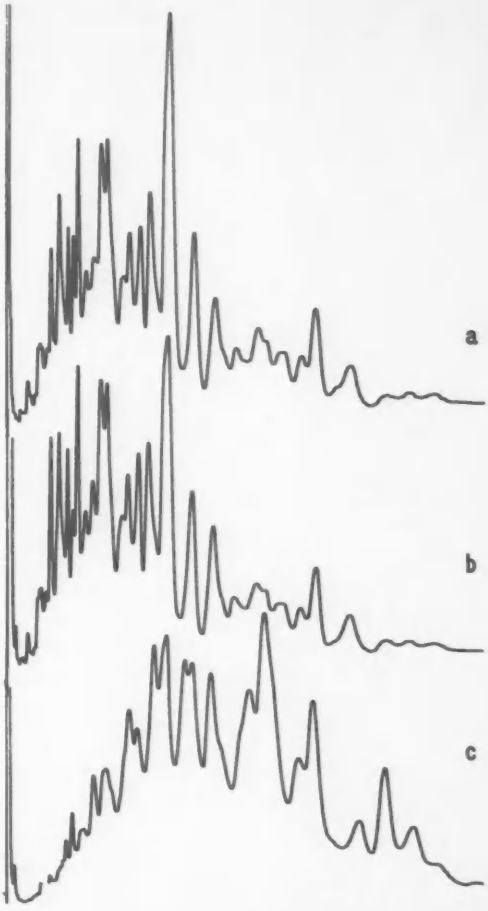

FIG. 1. (a, b) Chromatograms of two preparations of degraded toxaphene, 3% OV-225 column, 180 cm long × 0.3 cm inside diameter, 200°C isothermal, electron capture detection; (c) parent toxaphene standard.

Quantification of degraded toxaphene by EC−GC is uncertain because the response of an EC detector is not directly proportional to the number of chlorines in a molecule. For example, EC response factors (relative to mirex) for toxicant B and its two isomeric hexachlorobornane products from reductive dechlorination are 51, 13, and 23 (Saleh and Casida

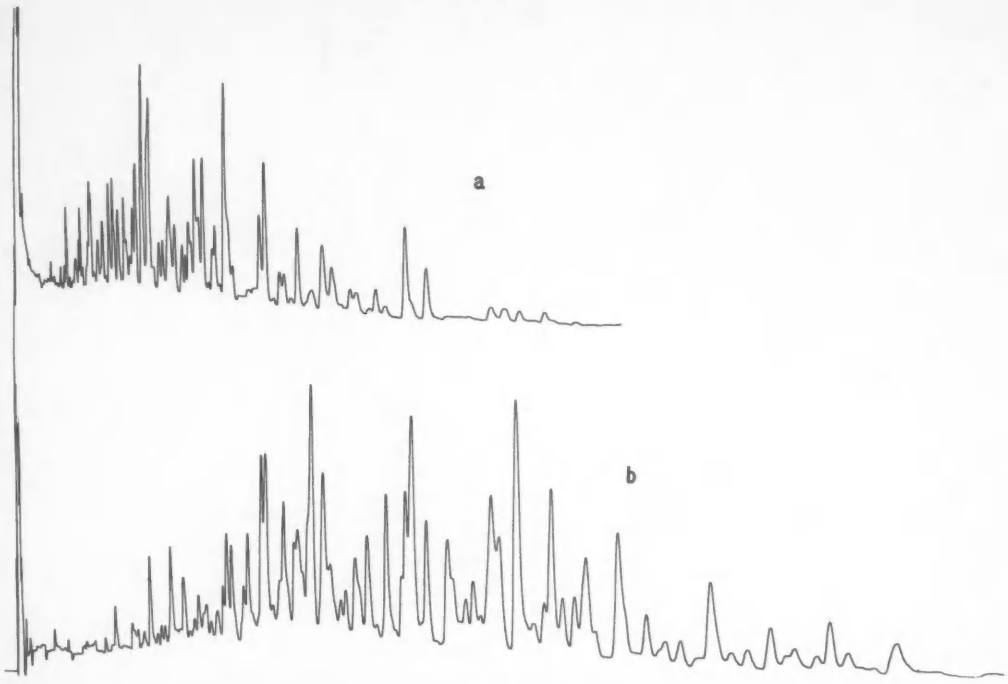

FIG. 2. Chromatograms of (a) degraded and (b) parent toxaphene, 30-m SE-54 glass capillary column, 180°C isothermal, hydrogen carrier gas, electron capture detection.

1978). In this case, response factors for the two products are lower than for the heptachlorobornane precursor. On the other hand, response factors for PCB isomers can vary by 5- to 10-fold, depending on the chlorine substitution position (Hutzinger et al. 1974). It is therefore difficult to generalize concerning EC response factors. Using the EC area response factor for parent toxaphene, the concentration of the degraded toxaphene stock solution was 2105 ± 126 μg/mL (six analyses). The degraded toxaphene concentration determined by FID–GC and EC–GC agreed well, considering the uncertainties involved, and the EC-determined concentration (2105 μg/mL) was used in calculating the bioassay system concentrations.

ACUTE TOXICITY TESTS

The results of the acute toxicity experiments with parent and degraded toxaphene are given in Fig. 3 for mullet and spot. The 96-h LC50 (±95% C.I.) for parent toxaphene to white mullet was 2.88 (2.51–3.31) μg/L. Butler (1963) measured a 48-h LC50 of 5.5 μg/L for this species in a flow-through system in which the toxaphene concentrations were not monitored. Correcting his LC50 for differences between monitored and unmonitored concentrations, and between a 96-h and a 48-h experiment (Federal Register 1978, 43: 21506–21508) gives an adjusted 96-h LC50 of 3.4 μg/L (EPA 1980), which agrees well with our result. Degraded toxaphene was almost three times more toxic to white mullet, with an LC50 of 1.02 (0.90–1.15) μg/L. The two toxicity values were significantly different ($P < 0.05$) according to the method of Litchfield and Wilcoxon (1949). This high toxicity was surprising, since all previous reports indicated that reductive dechlorination lessened the toxicity of toxaphene. Our results do not appear to be an artifact of the experiment. The rate of control survival was high, never exceeding 10% and usually 0%, and the agreement with the published LC50 for parent toxaphene was good. In addition, all LC50 values are the results of multiple bioassay experiments for each species and each toxicant concentration, and the agreement among similar runs was excellent (Fig. 3). The possibility of extractables from the sediment contributing to the higher toxicity of the degraded toxaphene is unlikely, as the controls survived at mud extract concentrations equivalent to three times those used in the degraded toxaphene experiments.

Further evidence of the toxicity of degraded toxaphene, although not as dramatic, are the results from the experiments with spot. Parent and degraded toxaphene LC50 for this species was 0.92 (0.83–1.02) and 1.10 (1.00–1.21) μg/L, respectively. Butler (1964) reported 1.0 μg/L as the 48-h LC50 for parent toxaphene to spot. Corrected as above, his adjusted 96-h LC50 becomes 0.62 μg/L (EPA 1980).

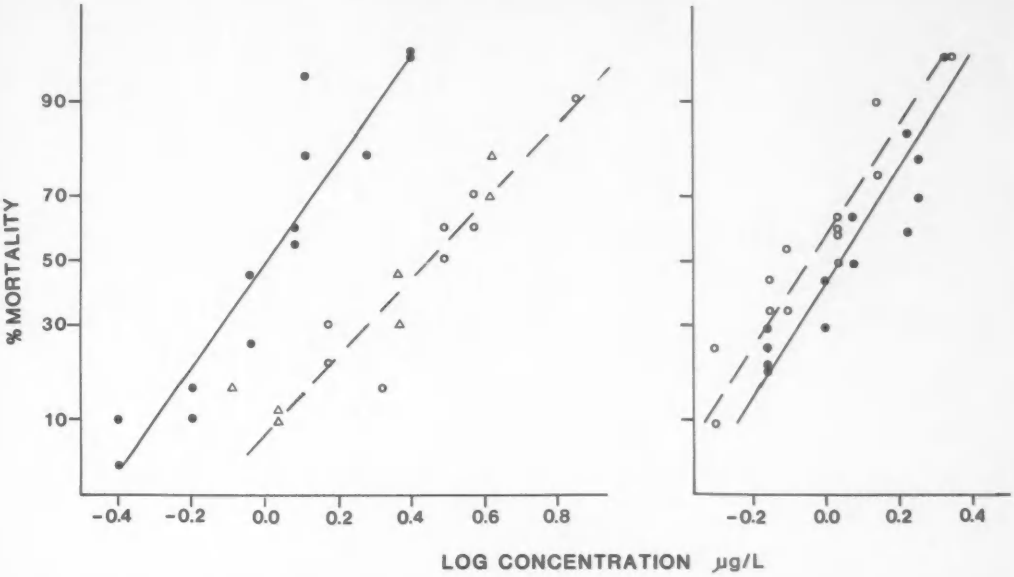

FIG. 3. Mortality of (left) white mullet and (right) spot exposed to parent toxaphene (open symbols) and degraded toxaphene (solid symbols). For the mullet experiments, open and solid circles represent experiments done with fish collected during January–February 1980. Triangles represent tests done with fish collected during February–March 1981.

TABLE 1. Bioconcentration factors (96-h) for parent and degraded toxaphene.

		Water concentration (µg/L)	Fish wet wt concentration (µg/g)	BCF
Parent toxaphene	Spot	0.7	2.9	4.1×10^3
		0.8	0.9	1.1×10^3
		2.4	8.4	3.5×10^3
	Mullet	0.7	4.0	5.7×10^3
		0.8	2.6	3.3×10^3
		2.4	10.4	4.3×10^3
		4.1	27.0	6.6×10^3
Degraded toxaphene	Spot	0.5	1.0	2.0×10^3
		0.8	3.7	4.6×10^3
		1.1	6.5	5.9×10^3
	Mullet	1.0	4.2	4.2×10^3
		2.2	13.3	6.0×10^3

Previous results with individual toxaphene components (Saleh et al. 1977; Saleh and Casida 1978) as well as with whole toxaphene applied to lakes (Lee et al. 1977) indicated that reductive dechlorination lessened toxicity, and it is interesting to speculate why our results are different. Saleh et al. (1977) observed relatively high toxicity for a few heptachlorobornanes and octachlorobornanes isolated from toxaphene and high toxicity for one hexachlorobornane. On the other hand, two isomers containing nine chlorines had low toxicities. Perhaps the enhanced toxicity of our sediment-degraded toxaphene resulted from a breakdown of highly chlorinated isomers to more toxic compounds containing fewer chlorines. A study of the distribution and toxicity of the individual components of sediment-degraded toxaphene would be helpful in answering this question.

BIOCONCENTRATION FACTORS

Individual fish exposed to various concentrations of toxaphene and degraded toxaphene were selected and analyzed for

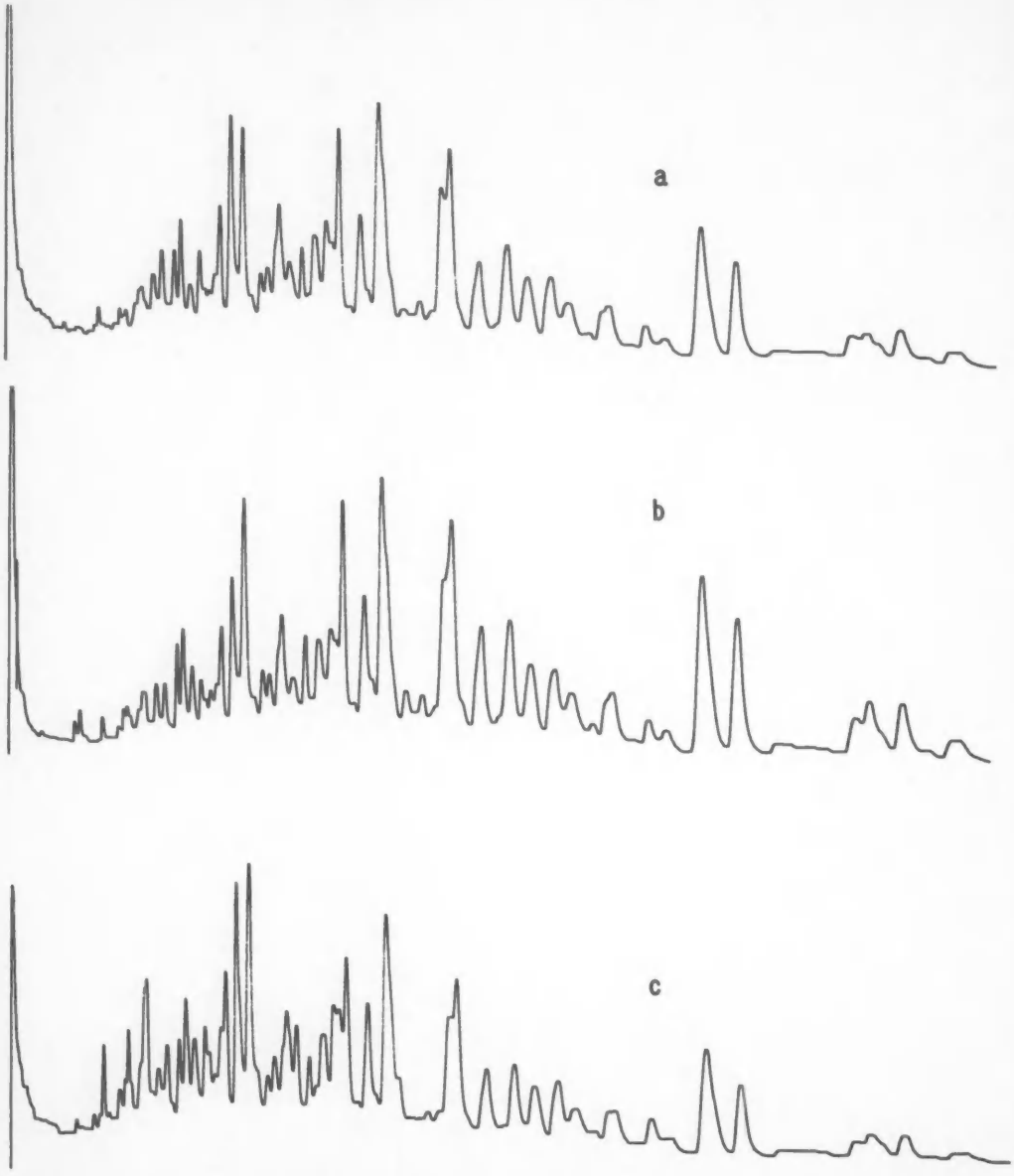

FIG. 4. Glass capillary chromatograms (30-m SE-54, 180°C isothermal, hydrogen carrier gas) of degraded toxaphene residues in fish from bioassay experiments. (a) Residues in spot exposed to 4 μg/L; (b) residues in white mullet exposed to 0.7 μg/L; (c) degraded toxaphene added to bioassay aquaria. Chart speed is about twice that in Fig. 2.

whole-body residues. The degree of accumulation was similar for both forms of toxaphene; 96-h BCF ranged from 2×10^3 to 6×10^3 for most fish tested (Table 1). These values are similar to published 96-h parent toxaphene BCF for two other estuarine fish, the sheepshead minnow (*Cyprinodon variagatus*, BCF = $3.1 \times 10^3 - 2.1 \times 10^4$) and the pinfish

(*Lagodon rhomboides*, BCF = 3.85×10^3) (Schimmel et al. 1977). Chromatograms of body burdens exhibited little difference from those of the respective water extracts, other than showing a slightly higher accumulation of the heavier components (Fig. 4). Toxaphene concentrations in control fish were less than 5 μg/kg wet wt.

Conclusions

Short-term degradation of toxaphene in anoxic sediments does not appear to reduce its toxicity; indeed, the degraded toxaphene produced in this work has as much potential for causing acute poisoning to fish as the parent pesticide. Since the toxaphene transformation products are not innocuous, it would be desirable to learn more about the rate of toxaphene reductive dechlorination in sediments, the sublethal effects of degraded toxaphene, and the distribution of toxic isomers in the mixture. Analytical searches for toxaphene residues in environmental samples should not be restricted to GC patterns matching those of the parent pesticide but should also consider the degraded toxaphene fingerprints.

Acknowledgments

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Sexual Maturity, Fecundity, Spawning, and Early Life History of Sablefish (*Anoplopoma fimbria*) off the Pacific Coast of Canada

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MASON, J. C., R. J. BEAMISH, AND G. A. MCFARLANE. 1983. Sexual maturity, fecundity, spawning, and early life history of sablefish (*Anoplopoma fimbria*) off the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. 40: 2126–2134.

Analysis of ichthyoplankton surveys and maturity states showed that sablefish (*Anoplopoma fimbria*) spawn along the entire Pacific coast of Canada from January through April with peak spawning occurring in February. Spawning took place at depths greater than 300 m all along the continental slope and did not entail a noticeable spawning migration. Fifty percent of females and males spawned for the first time at an age of approximately 5 yr. Length at 50% maturity was approximately 58 cm for females and 52 cm for males. The adult male to female ratio during the spawning seasons of 1980 and 1981 was approximately 1:3 and was 1:1.5 during all other sampling periods. The sex ratio of juveniles was 1:1. Fecundity estimates are described by the equation $F = 1.11987FL^{2.8244}$. After hatching in March and April, postlarvae moved into the surface waters and were found >180 km offshore in late March. Juveniles were found in inside waters in July and August, attaining a length of 9 cm by early August. Juveniles may remain in inside waters until maturity when they return to the spawning areas.

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Une analyse des relevés de l'ichtyoplancton et de l'état de la maturité démontre que la morue charbonnière (*Anoplopoma fimbria*) fraye tout le long de la côte du Pacifique du Canada de janvier à avril, avec sommet en février. La fraie a lieu à des profondeurs de plus de 300 m tout le long de la pente continentale et ne comporte pas de migration importante. Cinquante pour cent des femelles et des mâles frayent pour la première fois à l'âge d'environ 5 ans. La longueur à laquelle 50% des sujets sont matures est d'environ 58 cm chez les femelles et 52 cm chez les mâles. La proportion des mâles et des femelles adultes pendant les saisons de ponte de 1980 et 1981 a été approximativement de 1:3 et elle a été de 1:1,5 pendant toutes les autres périodes d'échantillonnage. La proportion des sexes des juvéniles était de 1:1. On peut décrire les estimations de fécondité à l'aide de l'équation $F = 1{,}11987LF^{2.8244}$. Après l'éclosion en mars et avril, les postlarves montent à la surface, et on les trouve à >180 km au large, fin mars. Les juvéniles se trouvent dans les eaux intérieures en juillet et août, atteignant une longueur de 9 cm au début août. Ils peuvent y demeurer jusqu'au moment de la maturité, alors qu'ils retournent sur les lieux de fraie.

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LITTLE is known about the spawning habits of sablefish (*Anoplopoma fimbria*). Fragmentary observations (Thompson 1941; Shubnikov 1963; Heyamoto 1962; Kodolov 1968, 1976; Bell and Gharrett 1945; Phillips and Imamura 1954) suggest that throughout the North Pacific Ocean sablefish may spawn from late fall to early spring. Thompson (1941) felt that spawning probably commenced in February off the Queen Charlotte Islands in northern British Columbia, based on description of eggs taken from a single ripe female and the presence of like eggs in plankton catches. Bell and Gharrett (1945) concluded that spawning occurred late in the year off Cape Flattery, based on accounts of ovarian states furnished by fishing captains. Phillips and Imamura (1954) stated that the spawning period off California was December through April with peak spawning occurring in January and February. In the Bering Sea, peak spawning was reported to be restricted to February (Shubnikov 1963) or to occur in the fall (Kodolov 1976). Larvae, postlarvae, and juvenile sablefish

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(11.3–30.2 mm TL) were taken off the Aleutian Islands (163°W to 175°E) in July and August by Kobayashi (1957). From the small size of some of these larvae, spawning in the general area may extend into late May.

The reported sizes and age ranges at 50% maturity vary and estimates often have not been recorded separately for each sex. Heyamoto (1962) indicated that 50% of a sample were mature at 48 cm and the males and females were mature at 56.0 and 67.5 cm, respectively. Age at 50% maturity was 5–7 yr. Kodolov (1976) reported a length range at 50% maturity of 60–64 cm and an age of 4–7 yr.

The Canadian fishery for sablefish developed rapidly over the past 5 yr, relative to traditional levels, and currently has the highest landed value in the groundfish fisheries of the Canadian Pacific coast, including that for Pacific halibut (*Hippoglossus stenolepis*).

Development of an effective management strategy for this important commercial species is dependent on reliable knowledge of age and size at maturity, timing, location, and relative magnitude of spawning, fecundity, and associated migratory behavior. These biological concerns are preliminary to elucidation of the stock recruitment mechanisms as they may relate to environment. Because available information was poorly documented and fragmentary, particularly off the North American coast, and there was no previous comprehensive study of reproductive biology, such a study was undertaken in 1979 and 1980 and included preliminary information on the early life history of sablefish referenced to environment.

Materials and Methods

Sablefish were examined for sex and state of maturity and were measured for fork length to the nearest centimetre. Pairs of otoliths were collected for age determination from subsamples of the total catch and stored in a 50% glycerin solution. The size of each subsample varied, but in all cases a portion of the total catch was sampled without selection. The method of age determination was described and partially validated by Beamish and Chilton (1982). Samples were collected from 18 cruises conducted between the period September 1979–May 1980 and a further cruise from 16 November to 20 December 1981. Data from two cruises conducted during the same period in 1978 have also been included in the analysis. Approximately 12 000 fish were measured, sexed, and examined for maturity states and 2684 of these fish were sampled for otoliths.

Two approaches were used to ensure that the timing and location of spawning were identified. Gonadal development was monitored from commercial catches at a number of locations along Canada's west coast, coincident with a late winter–spring series of egg and larval surveys throughout all commercially fished areas.

The gonad of immature females appeared as a thin, lobed thread of bubbly texture. The immature male gonad was similar but with no bubbly texture. Maturing and mature ovaries contained white, opaque eggs visible to the eye. Eggs became hydrated and translucent just prior to spawning. Maturing or mature males developed folds or lobes in the gonad of whitish coloration, and as the male matured, the number and size of the folds increased. Spawning females produced a stream of translucent eggs from the vent following slight external pressure on the body wall. The ovary from a spawned (or spent) female was flaccid, of purplish red color, and contained a few residual mature eggs. Males in spawning condition released sperm from the vent following slight external pressure on the body wall.

Lengths were grouped in 1-cm intervals and ages in 1-yr intervals. The median length and age at 50% maturity were identified using probit analysis (Leslie et al. 1945). Although this procedure provided median ages in fractional years, the median first spawning age is only meaningful biologically when recorded in whole years.

For determination of fecundity, ovaries of 220 females 58–110 cm FL were collected at sea from catches made during the 1st wk of February 1981. Fish were taken in Korean-type traps fished off the northwest coast of Vancouver Island and the west coasts of the Queen Charlotte Islands. Maturity state ranged from resting to spent ovaries, although most ovaries were ripe. Fifty-five ripe females were selected so that no more than three were in any centimetre interval. Fresh ovaries were preserved in 10% formaldehyde solution. In the laboratory, the preserved ovaries were transferred to Gilson's fluid, as modified by Simpson (1951), for 4 mo before extracting the eggs, to allow breakdown of connective tissue.

Ovaries were washed thoroughly in cold water over a series of stainless steel screens and gently broken up by hand to separate the hardened eggs from the ovarian tissue. The cleaned eggs were stored in 5% formaldehyde solution.

Eggs from a single ovary were transferred to a 20-L glass reservoir filled to either 10 or 15 L and stirred vigorously with a wooden paddle. A volumetric subsample of 25–50 eggs in 1–2 mL was extracted using Stempel pipettes. Fifty subsamples were transferred to petri dishes while stirring continued. Under the dissecting microscope at 50× magnification, all eggs were sized and counted in 20-μm-diameter intervals in five subsamples. These results were then combined to construct a size frequency histogram describing the ovary. The eggs larger than 500 μm in diameter were then counted in the remaining 45 subsamples to provide 50 subsample volumetric counts of large eggs. Without exception, all ovaries examined contained a bimodal distribution of egg size with peaks occurring at 100 and 1000–1200 μm. No difficulty was encountered in distinguishing between ripening, well-yolked eggs (large) and small eggs containing scant or no yolk material, and the two size-classes of eggs were separated by a consistent size gap of some 500 μm between 300 and 800 μm egg diameter.

The total number of large eggs was calculated from the product of mean subsample count of large eggs per millilitre and the reservoir volume prior to subsampling. From 1/300 to 1/100 of the total eggs in an ovary were actually counted.

Four monthly cruises were conducted from January to April 1980 to examine the distribution of sablefish eggs and larvae. The survey covered 77 stations located on 18 equidistant transect lines, which terminated seaward adjacent to the 500-fathom isobath (1 fathom = 1.83 m). Approximately 45 stations were situated on the 30-, 50-, 100- and 500-fathom isobaths of the outer continental shelf and slope.

At each station an oblique tow was made using 0.25-m^2

Bongo samplers (McGowan and Brown 1966) after the general sampling procedure described by Smith and Richardson (1977) with the following exceptions: all sampling components were black; nets were 351-μm Nitex and of modified SCOR design; codends were made of PVC with 351-μm stainless steel mesh windows; a mine sweeper depressor of approximately 100 kg was employed on the January cruise but replaced by a cylindrical weight (75 kg) on subsequent cruises. Along the outer shelf at tow depths exceeding 300 m, filtration volumes ranged from 400 to 800 m^3. Catches of eggs and larvae were standardized to number per 10 m^2 by applying a standard haul factor to the raw catches derived from the following equation:

$$\text{SHF} = \frac{\text{max. tow depth (m)}}{\text{volume filtered } (m^3)} \times 10.$$

Vertical profiles of salinity and temperature were obtained using a Bissett–Berman STD system, standardized from Nansen casts. Ichthyoplankton and hydrography data compilations are to be found in reports by Mason et al. (1981a, 1981b, 1981c, 1981d, 1981e, 1981f, 1981g).

Sablefish eggs are pelagic, 1.8–2.2 mm in diameter, and have a smooth, nondescript chorion. They are isolecithal with no oil globules, have a narrow perivitelline space, and the embryo remains unpigmented until the later stages (J. C. Mason, unpublished). The larvae were described by Kobayashi (1957).

Results and Discussion

Distribution and Timing of Reproduction

Male sablefish were found in spawning condition from late in the year until early spring. This ability of males to remain in prolonged spawning condition is common in fishes, rendering males unsuitable for defining the spawning period. In contrast, females were in spawning condition over a shorter time period, allowing recognition of a peak spawning period as a meaningful biological characteristic.

Intermittent weekly observations of female maturity state from a number of locations off the west coast of Canada (Fig. 1) indicated that females were in spawning condition from January through March with peak spawning occurring in mid-February (Fig. 2). Although it was logistically impossible to make daily observations simultaneously in all three major areas, the available daily samples (drawn predominantly from the Queen Charlotte Islands area) show that 50% of the females examined spawned by February 12 (Fig. 3). This date is accepted as being generally appropriate for all the major areas, suggesting no significant latitudinal effect. The small percentage of females reported in spawning condition in August, September, and November (Fig. 2) are of questionable significance and probably due to some naive observers incorrectly identifying ovaries as spent. Infrequently, however, fishermen have reported catching sexually mature sablefish at times outside the January to March spawning period so a few fish or some minor stocks may spawn outside the major spawning period.

Highest individual catches of sablefish eggs were taken in ichthyoplankton hauls made in January and February

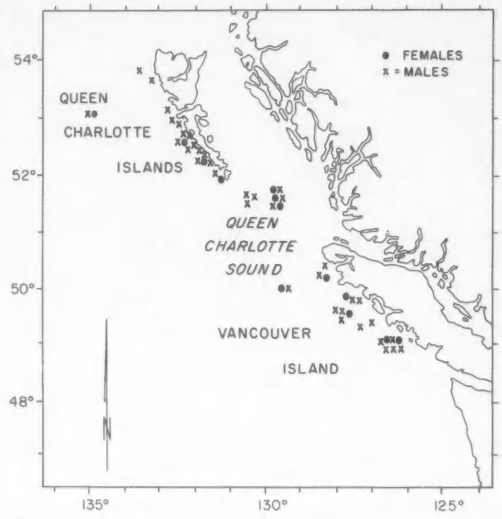

FIG. 1. Capture locations for spawning sablefish.

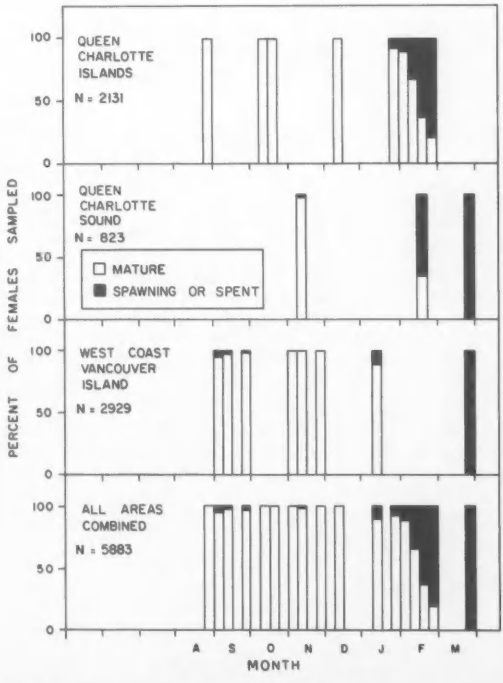

FIG. 2. Time of spawning in the Queen Charlotte Islands, Queen Charlotte Sound, Vancouver Island, and all areas combined.

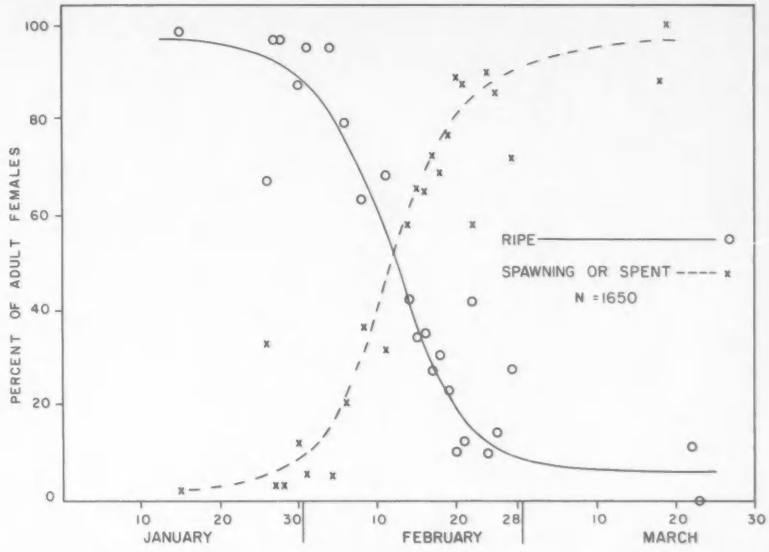

FIG. 3. Maturity states for female sablefish sampled daily between January and March on the British Columbia coast.

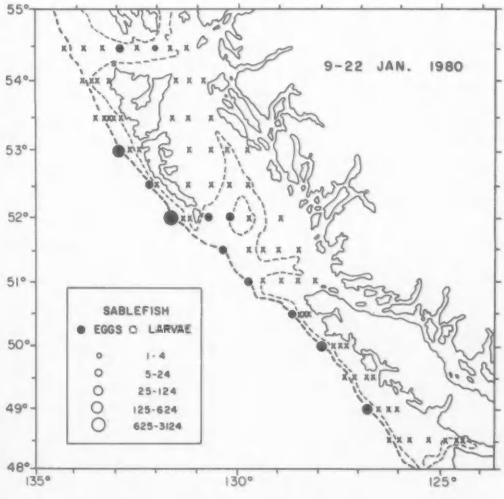

FIG. 4. Distribution and abundance (No./10 m^2) of sablefish eggs along the Pacific coast of Canada, January 1980. The 200- and 500-m isobaths are indicated by broken lines; × = zero catches.

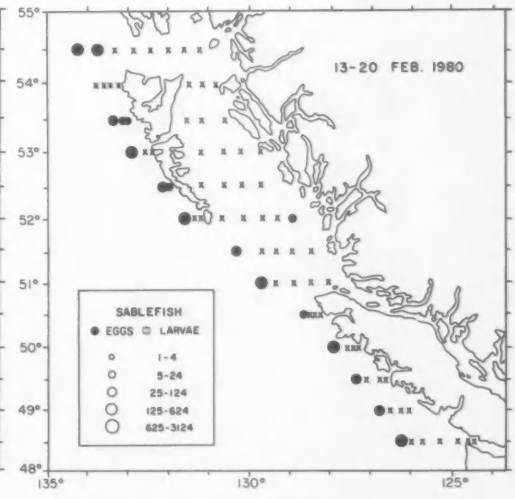

FIG. 5. Distribution and abundance (No./10 m^2) of sablefish eggs along the Pacific coast of Canada, February 1980. Symbols as in Fig. 4.

(Fig. 4–7) but eggs were taken in all four monthly cruises. The geometric mean catches (back-transformed means of the $\log_{10}$ catches) by cruise (Table 1) indicated peak spawning activity in February. The number of positive stations for larvae was greatest in April, suggesting a period of embryological development exceeding several weeks. The ichthyoplankton survey and the maturity survey both showed that spawning occurs almost simultaneously all along the coast (Fig. 2, 5). From the greater abundance of eggs in mid-February, the ichthyoplankton surveys (Table 1) indicate

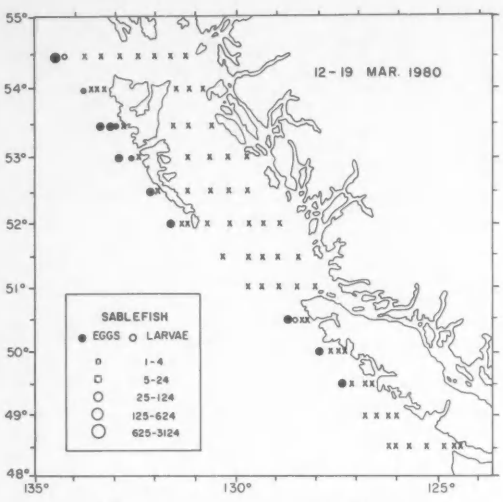

FIG. 6. Distribution and abundance (No./10 m^2) of sablefish eggs and larvae along the Pacific coast of Canada, March 1980. Symbols as in Fig. 4.

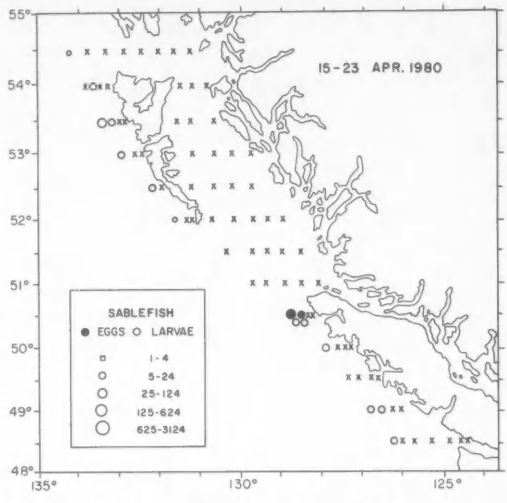

FIG. 7. Distribution and abundance (No./10 m^2) of sablefish eggs and larvae along the Pacific coast of Canada, April 1980. Symbols as in Fig. 4.

more spawning activity in late January than was indicated from the maturity observations. Since maturity observations were made on trapped fish, one might argue that such observations are biased toward feeding fish. However, our present data preclude effective testing of this hypothesis and we conclude that most spawning occurred during January and February with peak spawning occurring in February.

Our documentation of the seasonal timing of ovarian maturation and released egg production is similar to that reported by Phillips and Imamura (1954) for California stocks, suggesting the absence of a wide-scale latitudinal trend in reproductive timing although collection of larvae in the Aleutian Islands area in July and August (Kobayashi 1957) may reflect spawning activity there extending well into May.

The samples of spawning fish were obtained at depths ranging from 175 to 1450 m but most fish were caught at approximately 700 m, as this is an average fishing depth for commercial fishermen. The largest catches of both eggs and larvae were taken at stations where tow depth exceeded 400 m. Of 27 catches exceeding 25 eggs/10 m^2, 23 catches (85%) were taken at sampling depths exceeding 400 m. Similarly, the six highest catches of larvae were taken in tows deeper than 400 m, and 11 of 15 catches (73%) were made at tow depths exceeding 400 m.

Although the largest catches of eggs and larvae were taken west of the Queen Charlotte Islands, the small number of positive stations there (12 of 43 in all) does not allow the interpretation that spawning was more intensive (greater spawning stock) off these islands.

Almost all eggs were found at stations over or adjacent to the continental slope (Fig. 4–6). There was no indication of major spawning areas in Johnstone Strait, Hecate Strait, or Queen Charlotte Sound. Whereas fishermen have reported that small stocks of sablefish spawn in some mainland inlets,

TABLE 1. Geometric mean catches (No./10 m^2) of sablefish eggs and larvae from the Canadian Pacific Ichthyoplankton Survey, 1980. Values in parentheses indicate number of positive stations.

Sampling period	No. of stations occupied	Eggs	Larvae
9–19 January	72	31.1(12)	0(0)
12–17 February	77	78.9(17)	0(0)
11–16 March	77	43.6(12)	6.0(2)
15–20 April	77	20.3(2)	10.8(13)

there remains little doubt that the major spawning area is along the continental slope.

Because of the coastwide distribution of spawning activity, we suggest an absence of spawning migration, an interpretation that is supported by our tagging study. A total of 127 tagged sablefish were recovered during the maturity study (Table 2). These fish were tagged from February to September, 1977–80, as part of a program to identify sablefish stocks in the Canadian zone (Beamish and McFarlane 1983). Of 127 tags recovered, 90% were taken within 200 km and 81% within 100 km of the tagging location (Table 2) indicating that there is no extensive annual spawning migration, although localized movements of one or both sexes may occur.

All of the eggs captured were in the early or middle stages of embryological development. All 55 larvae taken were at least several days old, and 25 measurable larvae ranged in FL from 6 to 9 mm, the majority being 7–8 mm long. The noticeable absence of eggs in the later stages of development and a preponderance of newly hatched larvae (5–6 mm) are interpreted as evidence for an egg descent into waters exceeding 400–500 m (maximum depth of sampling) prior to hatching and maintenance of position at such depths at least

TABLE 2. Tagged sablefish recaptured from January to March 1980. Recoveries are segregated by distance from tagging sites.

Release period	Midpoint of release area	No. tagged	No. (%) of recoveries		
			<100 km	100–200 km	>200 km
July 1977	52°55′ 132°30′	5159	10	1	1
September 1977	43°30′ 126°20′	5506	12	—	—
May 1978	53°00′ 132°35′	5287	9	1	3
June 1978	51°20′ 130°05′ 50°15′ 128°20′	5465	16	—	—
May 1979	48°40′ 126°30′	8993	26	2	8
June 1979	53°00′ 132°35′	6300	26	7	1
August 1979	52°42′ 132°11′	281	2	—	—
February 1980	53°00′ 132°31′	1538	2	—	—
Total		38529	103(81%)	11(9%)	13(10%)

until hatching.

The lack of discrete-depth sampling gear disallowed any opportunity to document the actual vertical positions of eggs and larvae in the water column. Therefore, the actual depths of spawning, relative to the sea floor, equilibrium depths of egg stages, and the probable descent of eggs during development and ascent of larvae following hatching paralleling that suggested for the Pacific halibut by Thompson and Van Cleve (1936), must await further research.

AGE AND SIZE AT MATURITY

Mean lengths at 50% maturity were calculated for samples collected from four cruises from January to March 1980. The mean size of males varied very little among localities (Table 3). On the average, females were larger than males (56.8 and 52.5 cm, respectively). Mean ages at maturity were 4.8 for males and 5.1 for females indicating that 50% of males and females spawned for the first time at age 5. The second survey, conducted during November–December 1981, produced larger mean lengths for females at 50% maturity and sexual maturity for both sexes at a somewhat younger age.

The discrepancies in size and age at maturity between the two studies and among sampling areas probably reflect real variation and sampling error both in the field and in the ageing laboratory. For example, we know that sablefish grow very slowly in a particular area off the west coast of Vancouver Island (Beamish and Chilton 1982) and generally that faster growing marine fish tend to mature at younger ages (Beacham 1982, 1983). The method of age determination is difficult (Beamish and Chilton 1982) and ageing errors cannot be discounted. In 1981, larger members of the 1977 year-class matured and were recruited into the fishable stock. The inclusion of these fish in the 1981 sample tends to reduce average age at maturity and, since they were larger than 60 cm, to increase the average size at maturity. Thus, we have combined the 1980 and 1981 samples, resulting in average length and age at 50% maturity of 58.3 cm and 5.2 yr for females and 52.4 cm and 4.8 yr for males.

At present, a larger year-class (1977) is being recruited into the fishery. Approximately 50000 of these fish were tagged and released as juveniles. The recovery of tagged fish from 1981 to 1983 may reveal any tendency for fish to mature at larger sizes in northern latitudes and any variation in age at maturity associated with area of residency.

SEX RATIO

The male to female ratio of sablefish collected during prespawning and spawning periods in 1980 and 1981 (mid-November to mid-March) was 1:3. During all other sampling periods, the male to female ratio was 1:1.5.

Individual samples were quite variable. For example, in June 1980, of 1138 fish sampled off the Queen Charlotte Islands, 64% were male, whereas in June 1981 at the same location, a sample of 300 fish was 66% female. Of 412 sablefish collected during November and December 1981 from Queen Charlotte Islands, Queen Charlotte Sound, and Vancouver Island, females composed 64, 78, and 81% of samples, respectively.

Adult females dominate the commercial catch; however, it is doubtful that there are many more females than males in the population. Research samples of 412 and 200 juvenile sablefish collected in inside waters during September 1980 and August 1981 were composed of 50% males and females. Unless there is a differential mortality acting on adult fish

TABLE 3. Mean fork length[a] and age at 50% maturity of sablefish from (a) four cruises in January–March 1980, (b) one cruise bridging November–December 1981, and (c) combined data. Value in parentheses following mean value indicates sample size.

	Mean fork length (cm)		Mean age (yr)	
	♂ (*N*)	♀ (*N*)	♂ (*N*)	♀ (*N*)
Queen Charlotte Is. (52°N–U.S. border)	(a) 52.8(396)	56.6(1035)	4.5(248)	4.8(774)[b]
	(b) 51.6(240)	61.2(393)	4.2(163)[b]	5.6(276)[b]
	(c) 52.6(636)	56.6(1428)	4.6(411)	5.1(1050)
Queen Charlotte Sd. (50°50′N–52°N)	(a) 51.3(226)	57.5(349)	5.8(81)	6.0(158)[b]
	(b) 54.6(99)	63.4(702)	4.9(60)[b]	4.7(493)[b]
	(c) 51.9(325)	60.4(1051)	5.6(141)[b]	5.6(651)[b]
Vancouver Is. (N) (49°30′N–50°50′N)	(a) 55.6(164)[b]	62.0(95)[b]	6.5(36)[b]	6.0(52)[b]
	(b) 53.1(92)	61.7(415)	3.8(48)	4.6(234)
	(c) 53.1(156)	61.4(510)	3.5(84)	4.5(286)
Vancouver Is. (S) (U.S. border–49°30′N)	(a) 52.3(233)	56.0(609)	4.0(65)	5.3(187)[b]
	(b) —	—	—	—
	(c) 52.3(233)	56.0(604)	4.0(65)	5.3(187)
Weighted mean	(a) 52.5(919)	56.8(2083)	4.8(430)	5.1(1171)
	(b) 52.6(431)	62.4(1510)	4.3(271)	4.9(1003)
	(c) 52.4(1350)	58.3(3593)	4.8(701)	5.2(2173)
Mean	(a) 53.0(4)	58.0(4)	5.2(4)	5.5(4)
	(b) 53.1(3)	62.1(3)	4.3(3)	5.0(4)
	(c) 52.5(4)	58.6(4)	4.5(4)	5.1(4)

[a]To correct fork length to weight use the regression $W_g = 1.4 \times 10^{-6} \times FL^{3.5025}$ (Beamish et al. 1980) or $W_{kg} = 0.1923 \times 10^{-2} \times FL^{3.4638}$ (Kodolov 1976).
[b]Not determined by probit analysis.

after maturity, it is probable that the male to female ratio of the stock is equal and that the bias towards females encountered in commercial samples reflects selective trapping: relatively higher feeding or congregating activity of females, preferences of fishermen to catch larger fish, which are mostly females, etc.

FECUNDITY

Standard errors of the mean egg counts for fecundity estimates ranged between 0.6 and 4.0% of the means and were less than 2.5% in nearly 85% of the 55 counts. The variability of the enumeration technique compares favorably with that reported for Wiborg's whorling vessel (Wiborg 1951) as evaluated by Pitt (1963) for mature eggs of the American plaice (*Hippoglossoides platessoides*), which averaged 0.8 mm in diameter in Gilson's fluid. Pitt's test was applied to eggs from a single female and his standard error amounted to 2.2% of the general mean count drawn from 24 subsamples.

The fecundity estimates were regressed against fork length and increased with fork length where FL = fork length in centimetres according to the equation $F = 1.11987FL^{2.8244}$. The correlation coefficient (r) for the regression was 0.78. The smallest and largest females (57.9 and 115.0 cm) gave minimum and maximum fecundities of 58 200 ± 931 and 977 000 ± 16 400 maturing eggs. The average-sized female in the commercial fishery (70 cm) (R. J. Beamish, unpublished data) thus produces some 195 000 ripe eggs during the spawning season.

Although the scant information on fecundity reported in the literature cannot be compared extensively with our results, due in part to the lack of described quantitative methods, it is of some interest to consider it. Reported fecundities are higher than those presented here. The fecundity of three females taken in the Bering Sea by Kodolov (1968) is some 40–60% higher than for average females of equivalent size in this study. Females <80 cm in this study contained no more than 285 000 large eggs although two of Kodolov's fish (72.5 and 74 cm) had fecundities of 438 000 and 468 000 eggs, respectively. His third fish (82.5 cm) contained 503 000 eggs whereas the most fecund female <83 cm from Canadian waters contained only 485 000 eggs. Similarly, Kodolov (1976) reported a fecundity of 330 000 and 400 000 eggs for 70-cm females taken in the Gulf of Alaska and off Oregon–Washington, respectively, compared with 195 000 eggs (present study). Phillips and Imamura (1954) gave representative fecundities for California females of 100 000 and 1 million eggs for 21-in. (53.3 cm) and 40-in. (101.6 cm) lengths. The estimate for the smaller fish compares favorably with that derived from the present fecundity equation (90 303 eggs) but the larger fish, like Kodolov's, is some 55% more fecund than the average Canadian female of that size, as projected from the equation (641 416 eggs). However, the available data preclude an accurate comparison of larger fish due to their scarcity in the present study as well as in the existing literature. Only three females >90 cm FL were

available in the sample.

Early Life History

Our findings suggest that most sablefish spawn along the slope at depths generally exceeding 300 m. Egg descent during embryological development appears probable with hatching occurring predominantly at depths exceeding 400 m.

From January to late April 1980, average hydrographic conditions at depths from 300 to 750 m included temperatures ranging from 6.5 to 3.8°C and decreasing northward (a trend discernible despite local events occurring at depth), salinities ranging from 33.7 to 34.4‰, and sigma-*t* values ranging from 26.6 to 27.1, the last two parameters showing no latitudinal trends. Therefore, spawning, embryological development, hatching, and early posthatching life history stages take place below the relatively more dynamic upper waters, at temperatures and salinities generally below 6°C and above 34.0‰. Considering the minor hydrographic changes at depths exceeding 500–600 m with latitude, it is not surprising that a noticeable latitudinal trend in reproductive timing from California to Canada is absent.

After hatching, the young stages move into the surface waters and may be transported offshore or to shallow inshore regions. Four juveniles ranging from 21 to 35 mm were taken at the surface in late May, some 370 km seaward of Cascade Head, OR, by Brock (1940). Four larvae (9–10 mm) were captured in surface tows using a modified neuston sampler after Sameoto and Jaroszynski (1969) at two Line "P" stations some 70 and 175 km offshore in late March 1982 (J. C. Mason, unpublished data).

Juvenile sablefish have been observed recently in inside waters of British Columbia. On July 9, 1981, a series of surface tows using the neuston sampler caught nine juvenile sablefish in the entrance to Queen Charlotte Sound (51°37′N, 130°2′W). These juveniles ranged from 39 to 72 mm with an average length of 53 mm. Beamish and Chilton (1982) observed juveniles (5–9 cm) in Queen Charlotte Sound early in August. Despite the absence of major inshore surface currents during the summer months due to prevailing northwest winds producing offshore transport, passive onshore movement could occur in a countercurrent flow at the bottom of the mixed layer (100–150 m).

The 1977 age-class was found in great abundance that summer in Queen Charlotte Sound, Hecate Strait, and the mainland inlets (Beamish et al. 1980), indicating that large-scale onshore movement may occur subsequent to movement of the postlarvae into the surface waters.

These observations indicate that after hatching in March and April in British Columbia waters, sablefish larvae ascend to the surface and may be found as postlarvae at considerable distances offshore, well beyond the seaward boundaries of the spawning grounds (<55 km offshore). As juveniles, they can be found in inshore or inside waters at least by midsummer, possibly earlier, as some of the larvae captured on the outer shelf in April were taken shoreward of the egg distribution. The juveniles may remain inshore and in great abundance until they mature several years later. Preliminary observations (tag returns) of the movements of the abundant 1977 age-class indicate a return to the spawning grounds as adults.

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Many people assisted in the collection of the data used in this study and we sincerely appreciate their efforts. In addition to research staff, we particularly acknowledge the cooperation of commercial fishermen, who provided accommodation for our observers and returned tagged fish, and of the crews of the research vessels *G. B. Reed* and *Parizeau*.

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A Recurrent Red Tide in a British Columbia Coastal Lagoon

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ROBINSON, M. G., AND L. N. BROWN. 1983. A recurrent red tide in a British Columbia coastal lagoon. Can. J. Fish. Aquat. Sci. 40: 2135–2143.

The development of a recurrent fall red tide in Esquimalt Lagoon, British Columbia, has been monitored over an 8-yr period, including an intensive 1-yr field study. We suggest that growth of the organism, *Gymnodinium sanguineum* Hirasaka, which causes the tides, is regulated by the availability of nitrate supplied in runoff. Its decline is attributed to the seasonal decrease in the availability of light. The source of the seed population for these recurring blooms is unclear, although we propose that transport from California by the California Undercurrent is involved.

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On a suivi, durant une période de 8 ans, y compris une année d'études intensives sur le terrain, le développement d'une marée rouge automnale récurrente dans la lagune Esquimault, en Colombie-Britannique. Nous avançons l'hypothèse que la croissance de l'organisme *Gymnodinium sanguineum* Hirasaka, responsable de ces marées, est réglée par les nitrates apportés dans le ruissellement. On attribue son déclin à la diminution saisonnière de la lumière. On ne connaît pas très bien la source de la population reproductrice responsable de ces floraisons répétées, bien qu'à notre avis, le transport, depuis la Californie, par le courant sous-marin californien y soit pour quelque chose.

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RECURRENT red tides afford perhaps the best opportunity to study red tide dynamics. Not only can observations and experiments be repeated, but because of their predictability, recurrent red tides may be studied at all phases of their development, including the important early stages that are usually missed in studies of isolated red tide events. Significant advances have resulted from studies of recurrent phenomena (e.g. Steidinger 1975; Anderson and Wall 1978; Tyler and Seliger 1978, 1981; Tyler et al. 1982).

Some recurrent red tides, such as *Gonyaulax catenella* off the west coast of British Columbia, have not been studied in detail because of difficulties associated with tracking a population in open sea conditions, as well as problems of accessibility. For these reasons, red tide research on Canada's west coast has been restricted to reports of isolated outbreaks (Clemens 1935; Prakash and Taylor 1966; Quayle 1969).

We now report a recurrent red tide that occurs as a nearly captive population in a small British Columbia lagoon, making it an ideal candidate for studies of its dynamics and mode of recurrence. We present the results of an intensive (two samplings per week) study of the red tide through all phases of its development, conducted in 1978, as well as observations made over 8 yr of recurring red tides. Our objective was to examine the data and then to suggest ideas for the source of the red tides and their regulation.

Methods

In 1978 a detailed monitoring program was carried out in Esquimalt Lagoon, a small (approximately 2 km long and 0.8 km at its widest point), shallow (maximum depth 3.5 m), coastal lagoon situated near the southern end of Vancouver Island, B.C. (Fig. 1). It is almost completely landlocked, with the exception of a narrow channel at its northeast end that connects it with Juan de Fuca Strait. Tidal exchange through this channel is approximately 35% of the low-tide volume per tidal cycle (Scrimger 1960); however, this exchange is largely restricted to the waters at the mouth of the lagoon. Circulation is governed by tides and wind.

Sampling was conducted in the morning (08:00–10:00) twice weekly at three stations (Fig. 1) from July to December 1978, which encompassed the fall red tide. In situ measurements included submarine light intensity (400–700 nm) measured with a Kahlsico model 268WA310 underwater irradiameter and temperature and salinity measured using a Beckman RS5-3 portable salinometer. Water samples were collected by peristaltic pump at 1-m depth intervals and analyzed for chlorophyll *a* and phaeopigments (fluorometric determination), nitrate, nitrite, ammonium, and phosphate using the methods of Strickland and Parsons (1972). Integrated water samples (surface to 0.3 m off the bottom) were

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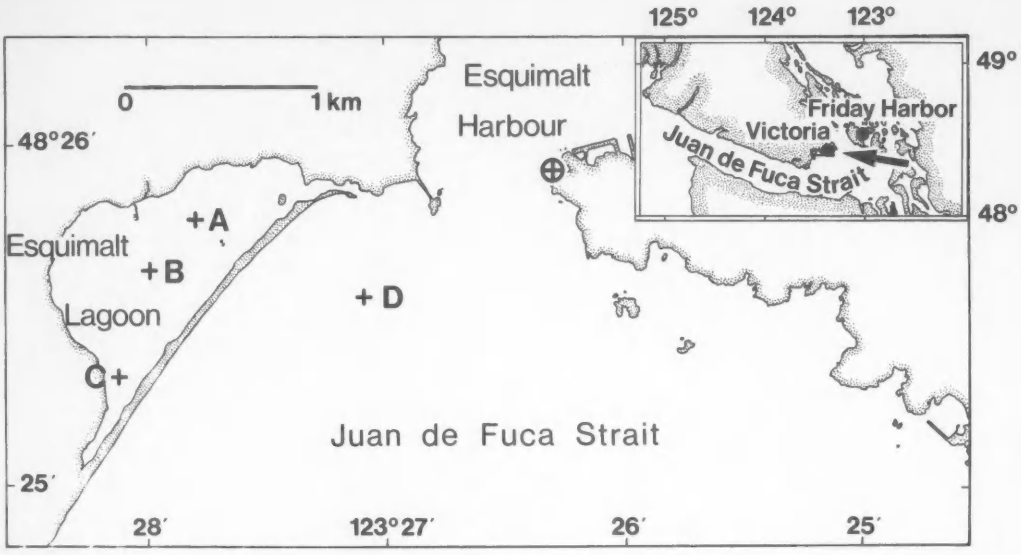

FIG. 1. Location of Esquimalt Lagoon and sampling stations A, B, C, and D. ⊕ indicates the location of the Esquimalt Harbour station used in 1982.

TABLE 1. Summary of samples taken from station B, Esquimalt Lagoon, 1974–77 and 1979–82. The intensive sampling program conducted in 1978 is described fully in the Methods section. Unless indicated, the methods used in all years were as described in Methods. a, temperature measured by thermometer; b, salinity samples collected by peristaltic pump and analyzed using an Autolab model 601 MkIII salinometer; c, chlorophyll *a* determined spectrophotometrically (Strickland and Parsons 1972) for samples integrated over the water column; d, phytoplankton concentrated by Millipore filtration, only *G. sanguineum* enumerated; e, only dominant species determined; f, samples collected from 1 m by peristaltic pump.

Year	Period of observation	Sampling frequency	Temperature	Salinity	Nitrate + nitrite	Phosphate	Chlorophyll *a*	Phytoplankton counts
1974	Oct. 14	Once	a	b	+	+	–	–
1976	June–Dec.	Biweekly or weekly	a	–	–	+	c	d
1977	Sept. 27	Once	–	–	–	–	–	e
1979	Jan.–June	1–2·wk^{-1}	+	+	+	+	+	+
	July–Aug.	Weekly	+	–	–	–	–	e
	Sept.–Nov.	Weekly	+	+	–	–	–	+
1980	June–Dec.	Weekly	+	+	–	–	–	+
1981	Sept.–Nov.	Weekly	–	–	–	–	–	f
1982	July–Nov.	Weekly	–	–	–	–	–	e

taken by lowering to depth a weighted length of 2.5-cm Tygon tubing vertically into the water, stoppering, and dispensing the contents into a collecting bottle. Samples so collected were analyzed for phytoplankton using qualitative, live examinations combined with quantitative counts of material fixed with Lugol's iodine (Utermohl 1958). Cell carbon (micrograms C per litre) was calculated from cell numbers and their measured geometries (Strathmann 1967).

Observations made at station B in 1974, 1976–77, and 1979–82 are summarized in Table 1. Except where noted, methods used were identical to those employed in 1978.

Results

The water column in Esquimalt Lagoon is generally stratified due to the presence of salinity gradients maintained by runoff from a number of small streams and tidal exchange with Juan de Fuca water. Wind occasionally produces mixing in the water column, particularly in winter; however, there is no persistent mixed layer. In 3 yr of periodic observations

TABLE 2. Summary of red water observations in Esquimalt Lagoon, 1974–82.

Year	Date first *G. sanguineum* cells observed	Red water[a]	Dominant species	*G. sanguineum* peak cell density (cells·mL^{-1})[b]	Date of peak
1974	+[c]	Oct. 14	—[d]		
1976	Aug. 10	Oct. 5, Nov. 16, Dec. 21	*G. sanguineum*	1 200	Oct. 5
1977	+[c]	Sept. 27	*Gymnodinium* sp.[e]		
1978	July 27	Sept. 14, Oct. 2–26 (Aug. 28–Nov. 6)	*G. sanguineum*	3 100	Oct. 23
1979	Aug. 27	Oct. 15 (Sept. 25–Oct. 22)	*G. sanguineum*	750	Oct. 15
1980	July 3	July 24, Aug. 6, 20 Oct. 22 (Oct. 1–22)	*Gymnodinium* sp. *G. sanguineum*	2 300	Oct. 22
1981	+[c]	(Aug.–Oct.)	*G. sanguineum*	11 400[f]	Oct. 20
1982	Aug. 30	Not observed	—[e]	~10[f]	Oct. 5–19

[a]Dates given in parentheses indicate periods during which water discoloration was weak or variable in intensity.
[b]Integrated over the water column.
[c]*G. sanguineum* well established at the time of first sampling.
[d]Dominant species not determined.
[e]*G. sanguineum* cells present, although not forming red water.
[f]Cell density at 1 m.

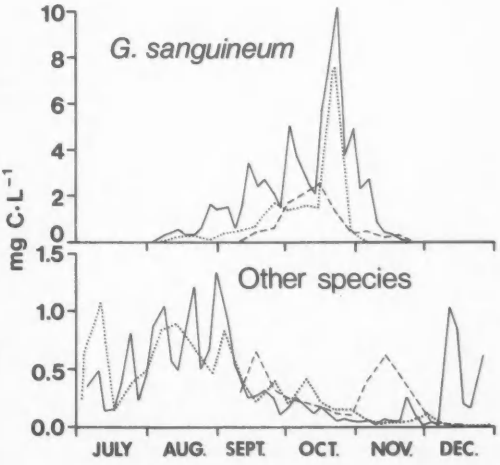

FIG. 2. Biomass of *G. sanguineum* and other phytoplankton species at station B, July–December of 1978 (—), 1979 (---), and 1980 (···).

(1978–80), salinities at station B were normally in the range 29–32‰, except following rain when salinity in the surface layer (<1 m) fell as low as 9‰.

Water temperature variations are large over both depth and time. Summer (June–August) values ranged from 11 to 21°C, with a 4–7°C surface-to-bottom gradient. Midfall (October) was characterized by isothermal conditions near 12°C. December temperatures ranged from freezing to 8°C, with an average of 6–7°C and a surface-to-bottom gradient of <1–5°C.

A major nutrient source to Esquimalt Lagoon consists of a small but steady input of nitrate from several small streams, which increases significantly following rainfall. Nutrient concentrations in the five major streams, measured between March 5 and June 25, 1979, averaged 54–74 μmol nitrate·L^{-1}, 0.57–14.8 μmol ammonium·L^{-1}, and 0.15–2.29 μmol phosphate·L^{-1}.

Light is strongly attenuated in Esquimalt Lagoon due to the presence of large concentrations of dissolved and particulate organic material. Dissolved organic carbon (DOC) and humic acid concentrations in the order of 2.5 and 0.5–2.0 mg·L^{-1}, respectively, were measured between April and August 1980 (A. H. Gillam, Herriot Watt University, Scotland, unpublished data).

CAUSATIVE ORGANISMS, DEVELOPMENT, AND DECLINE OF RED TIDES

A red tide was observed in Esquimalt Lagoon in 7 of the 8 yr during which observations were made (Table 2). Two organisms are apparently involved, *Gymnodinium sanguineum* Hirasaka (identified from a 1978 sample by F. J. R. Taylor of the University of British Columbia, Vancouver, B.C.) and a small (12–15 μm) unidentified *Gymnodinium* sp., the latter forming the dominant population on two occasions and the former dominating in all other instances in which species identifications were made. Importantly, *G. sanguineum* appeared every fall from 1976 to 1982, even when *Gymnodinium* sp. dominated the red tide or when no red tide was observed. Because *G. sanguineum* formed the red tide in the year during which detailed

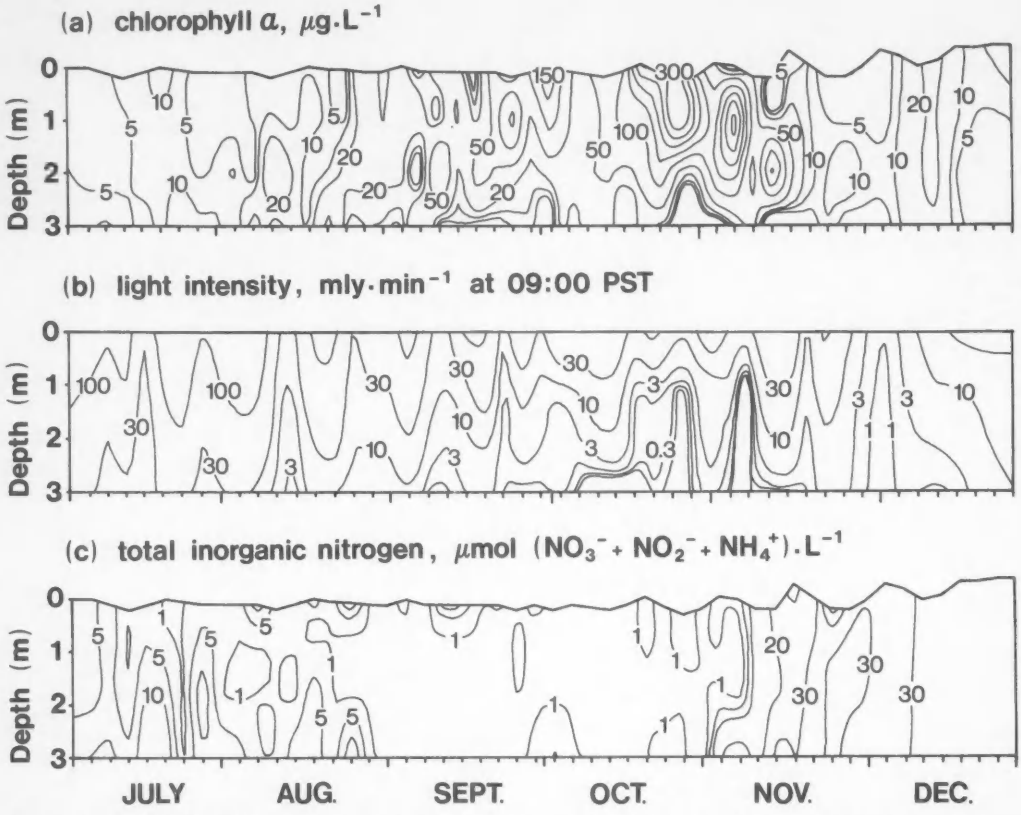

FIG. 3. Development of the 1978 *G. sanguineum* red tide in Esquimalt Lagoon (station B) showing light attenuation and nitrogen depletion produced by the red tide organisms.

investigations were made, and because our information on *Gymnodinium* sp. is limited, we choose to limit further discussion to the former species.

Figure 2 summarizes the growth of *G. sanguineum* in 1978, 1979, and 1980. Despite variations among years, the following general growth pattern can be identified: (1) the appearance of *G. sanguineum* cells in late summer or early fall (initiation), (2) its growth to a dominant position in the phytoplankton (bloom development), (3) the formation of a *G. sanguineum* monoculture and water discoloration (red tide), (4) a relatively rapid decline, and (5) the disappearance of *G. sanguineum* and the reappearance of other species (interbloom period).

The timing of the first appearance of *G. sanguineum* cells in our samples varied from early July in 1980 to late August in 1979 and 1982 (Table 2). We find no correlation between these dates and weather in any of the years studied (1976, 1978–80, 1982). Measurements of water temperature and salinity in 1978–80 revealed seasonal, generally stratified conditions at this time, with the exception of two wind mixing events in 1978. Phosphate and inorganic nitrogen concentrations measured in July 1978 were in the range 0.1–2 and 1–15 μmol·L^{-1}, respectively, reflecting generally reduced nutrient levels above 2 m but with pulses of nutrients accompanying wind mixing and runoff (Fig. 3).

The phytoplankton of the 1978–80 prebloom periods consisted of diverse communities of dinoflagellates and nanoflagellates, accompanied by short-lived (≤2 wk) blooms of diatoms, particularly members of the genus *Chaetoceros*, and occasionally flagellates. The only species that was associated with the appearance of *G. sanguineum* in all 3 yr was *Chaetoceros gracile* (49 000, 1100, and 82 000 cells·mL^{-1} at station B on July 24, 1978, September 10, 1979, and July 10, 1980, respectively).

The length of time between the initial appearance of *G. sanguineum* cells and the formation of red water varied from about 1.5 mo in 1979 to 2 mo in 1978 and 3.5 mo in 1980. In 1978, growth of *G. sanguineum* displayed a distinct periodicity that did not correlate with tide (mean tide height, volume of tidal exchange, or the time of day of lower low water). The pattern was, however, very consistent among stations (with the exception of the "white tide" at station C,

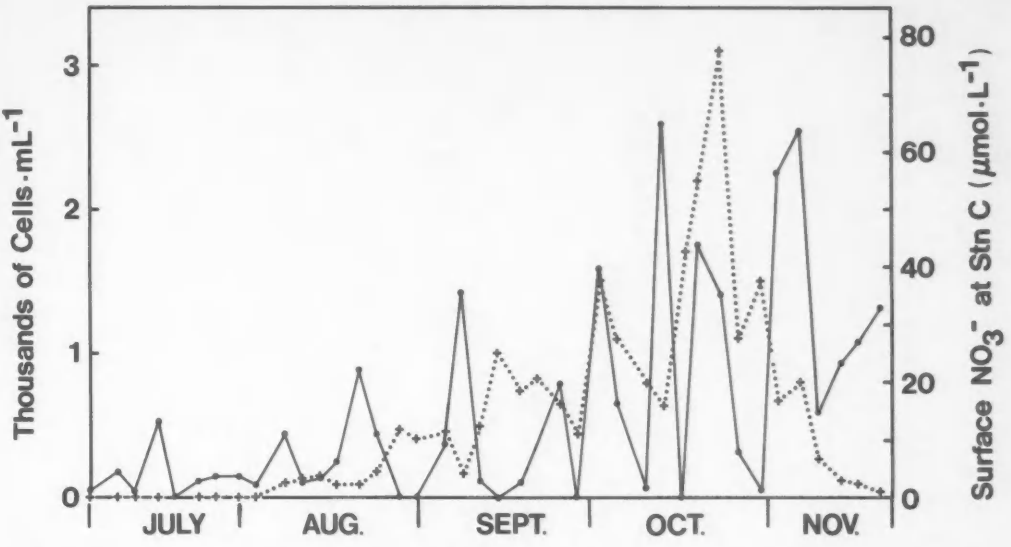

FIG. 4. *Gymnodinium sanguineum* abundance at station B (···) and nitrate from runoff (—, expressed as the surface concentration of nitrate at station C, which is located nearest a stream outlet) July–November 1978.

which is discussed later), suggesting that we were observing a real fluctuation in growth rate and not simply sampling error related to patchiness of the organisms.

In 1978, inorganic nitrogen concentrations decreased to <1 $\mu mol \cdot L^{-1}$ at all depths by late August (Fig. 3), with the exception of surface pulses of nitrate contained in runoff. These pulses correlated well with the observed fluctuations in growth ($P \approx 0.05$ for the correlation between nitrate concentration and *G. sanguineum* abundance, assuming a lag of 2.5 wk) and may have been at least partly responsible for regulating growth of the organisms (Fig. 4). Phosphate fluctuated with biomass, with 75% of the values in the range 0.5–1.5 $\mu mol \cdot L^{-1}$. No surface input from runoff was observed.

Unlike the later stages, the 1978–80 *G. sanguineum* blooms in their early weeks were accompanied by blooms of other species, notably the *Gymnodinium* sp. that formed red tides in 1977 and 1980, as well as diatoms including *Thalassiosira* spp. and *Rhizosolenia setigera*. In 1978 we noted a tendency for these blooms to follow pulses of runoff as previously observed for *G. sanguineum*. By early October (1978–80), blooms of other species were no longer observed, *G. sanguineum* forming 85% or more of the total phytoplankton biomass.

The most consistent feature of the *G. sanguineum* red tides has been the timing of the peak in cell numbers, in mid-October. This was true even in 1982, when red water was not observed. In contrast, the maximum observed biomass has varied considerably (Table 2). At their peaks, *G. sanguineum* red tides were essentially monospecific, forming 99.4, 93.6, and 98.0% of the total phytoplankton carbon in 1978, 1979, and 1980, respectively.

The color of *G. sanguineum* red tides was a deep blood red easily distinguished from the "tomato soup" color of the unidentified *Gymnodinium* sp. red tides. The intensity of the color varied with the depth of the organisms in the water column but was a clearly identifiable brown color at cell densities of about 400 cells $\cdot mL^{-1}$ and red at 1000 cells $\cdot mL^{-1}$. The organisms tended to form one or two broad patches that grew to extend over the entire lagoon.

The 1978 red tide effected several extreme changes in water quality. Light attenuation was severely reduced: the extinction coefficient in the top 1 m averaged 2.11 m^{-1} in October (Fig. 3). DOC concentrations increased nearly threefold from their prebloom levels to 7.2 $mg \cdot L^{-1}$ (A. H. Gillam, Herriot Watt University, Scotland, unpublished data). On one occasion (October 10, 1978), dissolved oxygen was depleted (<0.1 $\mu L \cdot L^{-1}$, measured with a YSI dissolved oxygen meter) from the entire water column at station C, which is located in a shallow (approximately 2 m deep), poorly flushed region of Esquimalt Lagoon. That morning, we observed clouds of "white tide," which turned the water a milky green color, in sharp contrast with the normal blood red color of a *G. sanguineum* red tide. The surface ammonium concentration increased from 1.4 $\mu mol \cdot L^{-1}$ on the previous sampling date (5 d earlier) to 13.9 $\mu mol \cdot L^{-1}$, strong sulphide odors were present, and benthic invertebrate mortalities were observed. Plankton samples contained abundant bacteria. By midafternoon the clouds of white tide were beginning to disperse, and by the following sampling day (3 d later) they had disappeared. Recurrences of these white tides were observed in bottom water at station C on October 19 and November 2, 1978. If, as suggested by Seliger et al. (1975), such transitory phenomena reflect the heavy nocturnal respiratory demands

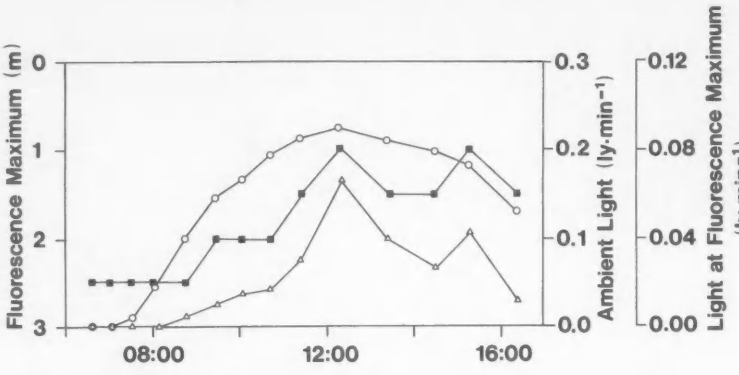

FIG. 5. Vertical migration of *G. sanguineum* 06:30–16:30 PST, October 9, 1979. ■, depth of fluorescence maximum; ○, ambient light intensity; △, light intensity at fluorescence maximum.

of red tide organisms, the cell densities recorded in 1978 may have approached the maximum attainable in a poorly circulated environment such as Esquimalt Lagoon.

We investigated the vertical migratory behavior of *G. sanguineum* during the 1979 red tide. Simultaneous measurements of in vivo fluorescence, ambient light intensity, and light intensity at the fluorescence maximum were made between 06:30 and 16:30 at station B on October 9. *Gymnodinium sanguineum* showed an upward migrating response when the light intensity at the fluorescence maximum exceeded 0.005 ly·min^{-1} (Fig. 5). The upward swimming speed was 0.5 m·h^{-1}, slower than that previously reported (1–1.7 m·h^{-1}) for *G. splendens* (≡ *G. sanguineum* ≡ *G. nelsoni* (Tangen 1979)) (Kiefer and Lasker 1975; Blasco 1979). We found no evidence of the predawn migration reported by Kiefer and Lasker (1975) for *G. splendens*.

The upward migration of *G. sanguineum* ceased when the organisms reached a depth of 1–1.5 m. (This is somewhat deeper than suggested by 1978 data: midmorning profiles of chlorophyll *a* frequently showed maxima in the 0- to 1-m depth range (Fig. 3).) The light intensity at that depth was 0.068 ly·min^{-1}. This is consistent with the findings of Cullen and Horrigan (1981), who reported that *G. splendens* forms subsurface layers at a light intensity at which photosynthesis becomes saturated, about 250 μE·m^{-2}·s^{-1} (or about 0.078 ly·min^{-1} according to Hollaender 1956).

In 1978–80 the disappearance of all traces of discoloration from lagoon water occurred rather quickly, following 1–3 wk after the peak in *G. sanguineum* biomass. In 1978 and 1979 a small population (approximately 10 cells·mL^{-1}) lingered until mid-December and late November, respectively. For 1976, however, we have evidence of discolored water as late as December 21, with cell densities of 390 cells·mL^{-1} at station C. It is not known how long *G. sanguineum* persisted in the lagoon that year.

Measurements in 1978 showed a rapid increase in nitrate and ammonium concentrations in the first 3 wk of November, from 1 to 20 and 8 μmol·L^{-1}, respectively. A progressive deepening of the chlorophyll maximum was observed, suggesting a possible deterioration in the migratory behavior of the red tide organisms or, perhaps more likely, a delay in their morning upward migration associated with a progressively later sunrise (Fig. 3).

In 1978 and 1980, *G. sanguineum* remained the dominant phytoplankter throughout the decline period; however, in 1979, two species of the euglenoid, *Eutreptiella*, bloomed (1200 and 460 cells·mL^{-1}) during this period.

To determine whether active *G. sanguineum* cells persist in Esquimalt Lagoon throughout the year, we did net tows (28-μm mesh) in July and August 1979 and January and March 1981. We were unable to detect any cells of this species (detection limit about 1 cell·m^{-3}) in any of the tows, suggesting that the mechanism for its yearly recurrence is unlikely to be seeding from an endogenous population.

SOURCE OF CELLS

If *G. sanguineum* does not persist in Esquimalt Lagoon in its vegetative state, then the seed population must arise either from an endogenous dormant population (cysts) or from an external source. We conducted initial investigations into both of these possibilities.

Numerous dinoflagellate hypnocysts have been isolated in our laboratory from sediments collected (by peristaltic pump) from Esquimalt Lagoon and areas immediately adjacent to its mouth, and from the water column (using sediment traps), throughout the year and especially during and after a *G. sanguineum* red tide. But all attempts to induce both untreated sediment and isolated cysts to excyst in the laboratory (using methods adapted from Wall et al. 1967 and Anderson and Wall 1978) and to produce viable *G. sanguineum* have failed. We have also failed to induce hypnocyst formation by nutrient and temperature stress, although *G. sanguineum* readily "rounds up" in the laboratory to form temporary or lab cysts, as has been observed for other dinoflagellates (Dale 1977).

During routine examinations of both natural and cultured samples of *G. sanguineum* we have noted a number of aber-

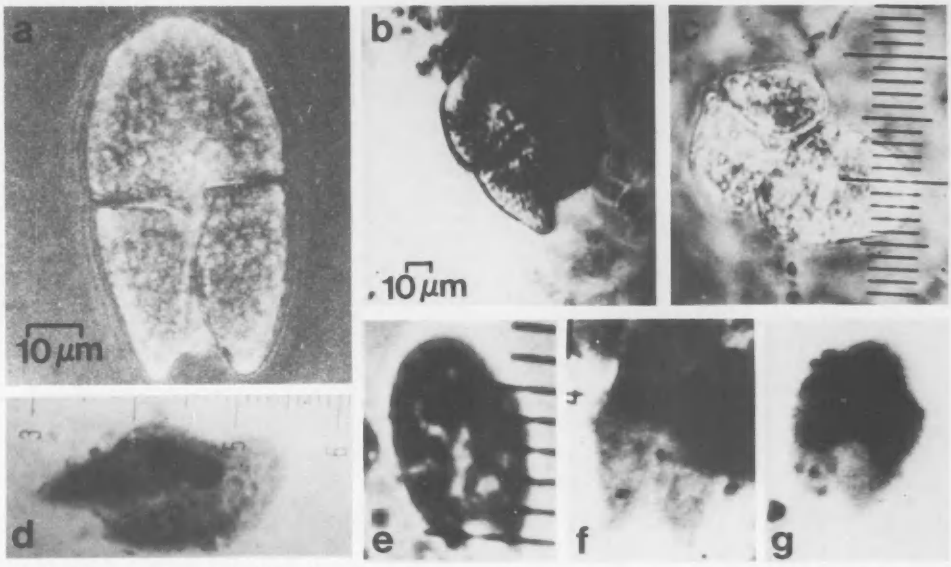

FIG. 6. Possible sexual stages in the life cycle of *G. sanguineum*. a–c, live; d–g, haematoxylin stained. a, vegetative cell; b, c, possible fusions; d, normal oblique fission; e–g, possible sequence of nuclear fusion. Note the bilateral plane of "fusions."

rant cells. Some of these appear to be sexual stages, possibly gamete fusions (Fig. 6b, 6c). We have looked for further evidence of cell fusion in fixed samples from the 1978 red tide using Harris' haematoxylin (eosin method). Figure 6d shows the haematoxylin-stained nucleus of a normal cell undergoing fission and Fig. 6e–6g show what may be cellular and nuclear fusion. However, we have as yet no evidence for the transformation of these "stages" into hypnocysts.

The waters immediately adjacent to Esquimalt Lagoon (Fig. 1, station D) were sampled twice in 1978, once just prior to the appearance of *G. sanguineum* in the lagoon (July 24) and again during the red tide (October 17), from depths of 0–10 and 0 m, respectively; *G. sanguineum* was not detected in either case. In mid-July 1982, a weekly sampling program was initiated at the entrance to Esquimalt Harbour, 1 km east–northeast of the entrance to Esquimalt Lagoon (Fig. 1), and at station B in the lagoon. On August 27, several cells of *G. sanguineum* were identified from Esquimalt Harbour and the first cells were noted at station B on August 30. The occurrence of *G. sanguineum* coincided with its appearance in moderate numbers at Friday Harbour, Washington, 35 km east–northeast of Esquimalt Lagoon (F. J. R. Taylor, University of British Columbia, personal communication).

Discussion

Regulation of Bloom Growth

A red tide has been described as the final phase of phytoplankton succession where stability of the water column is maintained and there is a continuous, or at least periodic, supply of nutrients (Margalef 1962; Davis 1982). These conditions are adequately met in Esquimalt Lagoon, and so it is not entirely unexpected that a red tide should develop almost every year. The reason for the predominance of *G. sanguineum* over *Gymnodinium* sp. is not yet clear.

The initiation of *G. sanguineum* blooms has demonstrated no clear relationship with weather or the physical or chemical properties of the water. It is unlikely that either humic substances or preconditioning by other algae are involved. Moderate concentrations of humic substances were found year-round in Esquimalt Lagoon; also, we have found that *G. sanguineum* grows well in culture media lacking chelators such as EDTA. Among the algal species that preceded *G. sanguineum* in 1978–80, only *C. gracile* was observed in all 3 yr, but filtrates from cultures of this species have been found to have no stimulatory effect on the growth of *G. sanguineum* (M. G. Robinson and L. N. Brown, unpublished data). Likely, a clearer understanding of factors regulating the initiation of the blooms will be possible when the source of the organisms is known.

Evidence from the 1978 study suggests that the growth of the *G. sanguineum* bloom was regulated by the nitrate supplied in runoff. It has also been suggested (e.g. Eppley and Harrison 1975) that downward nocturnal migration in some dinoflagellates enables them to utilize nutrients released from the sediments. Since *G. sanguineum* does appear to concentrate near the bottom at night, this may have provided a secondary source of nutrients that sustained the population in the periods between pulses of runoff. Further, we suggest that the ultimate magnitude of the bloom each year is directly related to the nutrient support available during the growth

phase. We observed a direct correlation between the magnitude of the red tide in 1978–80 and 1982 (the years for which complete data are available) and the total rainfall during the growth phase (between the initial appearance of the cells and the peak in biomass), with a correlation coefficient of 0.93.

From our data we also suggest that *G. sanguineum* is able to displace virtually all other members of the phytoplankton, with the possible exception of the red tide-forming *Gymnodinium* sp. observed in 1977. (Since a single observation was made that year, it is not known whether *G. sanguineum* later displaced *Gymnodinium* sp.) Data from the 1978 study suggest that depletion of nitrate or shading by *G. sanguineum* in a stable water column may be involved (see Fig. 3). The large increase in DOC concentrations suggests a possible release of a chemical inhibitor by senescent cells (cf. Kayser 1979). However, *Eutreptiella* spp. were able to bloom during the decline of the 1979 red tide; as well, we have obtained good growth of *Thalassiosira* sp., *C. gracile*, and *G. catenella* in filtered, nutrient-enriched lagoon water collected during the decline of the 1980 *G. sanguineum* red tide.

Oxygen starvation brought on by high cell densities has been implicated in white tide mortalities of *G. sanguineum*; the factor that causes the ultimate decline of these blooms, however, appears to be seasonal rather than density dependent. This is evidenced by the regularity in timing of the peak in biomass, independent of its magnitude. The exceptional persistence into late December of red water in 1976 coincided with exceptional weather conditions: although temperatures were near normal, the hours of bright sunshine during the period September–December that year set a 40-yr (high) record. We therefore suggest that the seasonal decrease in light mediates the decline of *G. sanguineum* blooms in Esquimalt Lagoon.

Source of Cells

The regularity with which *G. sanguineum* appears in Esquimalt Lagoon suggests seeding by hypnocysts. Overwintering cysts are believed to provide seed populations for other recurrent red tides (e.g. Steidinger 1975; Anderson and Wall 1978), but we have found no evidence of *G. sanguineum* cysts in Esquimalt Lagoon or adjacent waters. Moreover, Anderson and Morel (1979) suggested that temperature plays a critical role in the excystment of *Gonyaulax* hypnocysts, but we have found no correlation between the appearance of *G. sanguineum* in Esquimalt Lagoon and bottom water temperatures in 1978–80.

An alternate possibility is seeding by transport from an external population, as suggested for *Prorocentrum mariae-lebouriae* in Chesapeake Bay (Tyler and Seliger 1978). The simultaneous occurrence of *G. sanguineum* at Friday Harbour and in Esquimalt Lagoon in 1982 would support this hypothesis. Since both locations adjoin Juan de Fuca Strait, it is tempting to speculate that *G. sanguineum* may be seeded from a common source. One possibility is the chlorophyll maximum layer off California, of which *G. splendens* is a common component and has been known to form a red tide (Kiefer and Lasker 1975; Cullen and Renger 1979; Cullen and Horrigan 1981). Upwelling of California Undercurrent water occurs annually during June–November off the entrance to Juan de Fuca Strait (Freeland and Denman 1982). *Gymnodinium nelsoni* was observed in early August 1980 at several stations in this area (K. L. Denman, Institute of Ocean Sciences, Sidney, B.C., personal communication).

Estuarine circulation in the strait transports coastal water from this region into the inner strait where turbulent mixing brings it to the surface (Herlinveaux and Tully 1961). If the California Undercurrent does indeed transport *G. sanguineum*, then it must be carried along as a resting stage, since the water is brought up from approximately 450 m (Freeland and Denman 1982). Further research on the life cycle of *G. sanguineum* is required.

Acknowledgments

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Similarities in Avoidance Temperatures of Freshwater Fishes

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A statistical analysis of new and published data revealed strong geographic similarities in laboratory-determined avoidance temperatures of several freshwater fishes. Validated regression models developed from our laboratory data predicted avoidance temperatures, within specified variation, of species from other areas. Four cyprinids, two ictalurids, seven centrarchids, and two percids were tested. Species within each family showed similar avoidance temperatures. An exception was white crappie, *Pomoxis annularis*, which avoided lower temperatures than other centrarchids. Acclimation temperature was the most significant predictor variable. No significant differences of practical value were observed in avoidance temperatures between rising and falling field temperature. Our analysis provides an impetus to minimize site-specific laboratory studies of temperature avoidance for individual species and provides an objective method for literature data comparisons.

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Une analyse statistique de données nouvelles et publiées montre de fortes ressemblances géographiques des températures d'évitement de plusieurs espèces de poissons dulçaquicoles mesurées en laboratoire. Grâce à des modèles de régression, dont la validité a été confirmée, élaborés à partir de nos données de laboratoire, on a pu prédire les températures d'évitement, dans les limites d'une variation déterminée, d'espèces provenant d'autres régions. Nous avons expérimenté sur quatre cyprinidés, deux ictaluridés, sept centrarchidés et deux percidés. Au sein de chaque famille, les espèces montrent des températures d'évitement semblables. Il y a toutefois exception avec la marigane blanche, *Pomoxis annularis*, qui évite des températures plus basses que celles des autres centrarchidés. La variable prévisionnelle la plus significative est la température d'acclimatation. On n'observe aucune différence significative de valeur pratique des températures d'évitement entre des températures en hausse ou en baisse sur le terrain. Notre analyse nous incite à minimiser les études en laboratoire, spécifiques au site, des températures d'évitement d'espèces individuelles et elle nous fournit une méthode objective pour comparer les données publiées.

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IN earlier papers (Mathur and Silver 1980; Mathur et al. 1981, 1982) we provided statistical analysis of published and new data on laboratory-determined acute temperature preferences of several freshwater fishes and a crayfish, *Orconectes obscurus*. A strong evidence of similarity in these data among species within a family and among geographical populations was revealed. Biological significance was distinguished from statistical significance based on sample size and statistical power of those tests. In the present paper, available data on another laboratory thermal behavioral response, avoidance temperatures of warmwater fishes, are analyzed. The objectives are to determine if (1) regression models developed from

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TABLE 1. Regression statistics for the relationship of avoidance temperature (Y) and acclimation temperature (X) of Muddy Run–Conowingo (Pennsylvania) fishes. Standard error of estimates (SE) used for setting 90% confidence intervals around the regression and R^2 is the coefficient of determination.

Species	N	R^2	SE	Intercept (a)	Slope (b)	Range of acclimation temperature (°C)	Range of total fish length (mm)
Cyprinidae							
Spottail shiner, *Notropis hudsonius*	10	0.88	2.42	12.46	0.73	6.1–28.3	40–136
Swallowtail shiner, *Notropis procne*	52	0.87	2.39	15.10	0.69	0.0–28.9	47–61
Spotfin shiner, *Notropis spilopterus*	141	0.68	3.61	14.78	0.62	0.0–28.3	44–94
Bluntnose minnow, *Pimephales notatus*[a]							
Falling	39	0.67	4.09	12.22	0.77	0.6–27.2	44–68
Rising	24	0.80	3.13	14.93	0.80	1.1–25.0	44–75
Ictaluridae							
Brown bullhead, *Ictalurus nebulosus*	26	0.41	3.39	15.39	0.68	13.9–26.7	48–257
Channel catfish, *Ictalurus punctatus*	82	0.64	4.89	14.31	0.74	0.6–32.2	50–250
Centrarchidae							
Redbreast sunfish, *Lepomis auritus*	18	0.66	3.02	15.01	0.71	10.0–28.9	56–162
Green sunfish, *Lepomis cyanellus*	19	0.67	3.80	19.34	0.59	3.3–26.1	40–99
Pumpkinseed, *Lepomis gibbosus*	70	0.73	3.57	16.36	0.71	1.7–32.2	47–136
Bluegill, *Lepomis macrochirus*	94	0.52	3.13	23.76	0.38	1.1–32.2	28–137
Smallmouth bass, *Micropterus dolomieui*	30	0.65	2.25	20.14	0.51	10.0–32.2	55–118
Largemouth bass, *Micropterus salmoides*	132	0.75	3.14	17.69	0.62	1.1–33.9	49–153
White crappie, *Pomoxis annularis*	90	0.72	3.08	15.23	0.62	1.1–28.9	65–168
Percidae							
Yellow perch, *Perca flavescens*	48	0.62	2.59	17.82	0.54	5.0–29.4	38–120
Walleye, *Stizostedion vitreum*	35	0.41	3.79	20.58	0.42	0.0–27.8	70–120

[a]See Table 2 for analysis of combined data.

our studies conducted in southeastern Pennsylvania can predict avoidance temperatures of fishes in other areas, (2) significant differences occur when ambient field temperatures rise or fall seasonally, and (3) species within a family respond similarly. The results of our analysis, particularly when coupled with those reported earlier, should yield substantial savings in time and money and may reduce subjectivity in the interpretation and comparison of data from several sources in these types of studies.

Methods

Fishes for our study were collected throughout the year from Conowingo Pond, Pennsylvania–Maryland, and Muddy Run Pumped Storage Pond, Pennsylvania, by seine, trap net, and block net from November 1972 through April 1978. Fishes that could not be obtained by the above methods were purchased from hatcheries. These collection methods were designed to impose minimal stress on the fishes. On several occasions certain species were also captured in 2-min hauls of a 4.9-m semiballoon trawl. Fishes were usually held at the collection (acclimation) ambient temperature for 12–96 h before testing.

The design of the avoidance apparatus and the testing procedures have been described by Meldrim and Gift (1971) and Peterson and Schutsky (1976). Statistical problems exist in avoidance temperature studies because of a lack of independence of observations (Mathur et al. 1982). As a consequence, experimental trials in avoidance temperature studies (Cherry et al. 1975, 1977; Stauffer et al. 1976) were terminated as the result of the improper use of analysis of variance or t-tests. At the time the studies were conducted those were the prevalent procedures used to determine when avoidance temperature trials should be terminated (Mathur et al. 1982).

Statistical Analysis

The methods of data analysis used herein are similar to those given in Mathur et al. (1981, 1982). A predictive and verifiable statistical model for fishes tested in our laboratory was developed to describe the relationship between the avoidance temperature (Y) and acclimation temperature (X_1), fish length (X_2), light level (X_3), acclimation time (X_4), and pH (X_5) for each species and species grouped within a family. Data for the periods of rising (winter to summer) and falling (summer to winter) field temperatures were initially analyzed separately. If no significant differences ($P > 0.01$) were observed a new validated regression model was developed for pooled data.

Regression models that explained the largest portion of the variance (R^2) and had the smallest standard error were accepted for prediction. No variable was included in the final regression equation unless it was significant at $P \leq 0.01$, though the variables were allowed to enter the model at the $P = 0.50$ level. Because the presence of large numbers of degrees of freedom in the error term makes microscopic

TABLE 2. Covariance analysis of avoidance temperatures: acclimation temperature regressions for fishes tested during seasons of rising and falling temperatures in Muddy Run–Conowingo (Pennsylvania). Differences between elevations and slopes of seasonal regressions were rejected for $P > 0.01$ or when the amount of variance explained was small.

		% of total sum of squares			Probability	
	N	Acclimation temperature (X)	Intercept (a)	Slope (b)	Intercept (a)	Slope (b)
Swallowtail shiner	52	87.1	0.1	0.1	0.89	0.44
Spotfin shiner	141	67.6	0.4	0.1	0.19	0.89
Bluntnose minnow[a]	63	68.2	4.4	0.2	<0.01	0.83
Brown bullhead	26	41.3	1.7	8.8	0.39	0.06
Channel catfish	82	64.4	0.1	0.2	0.60	0.47
Pumpkinseed	70	72.8	0.8	0.1	0.16	0.55
Bluegill	94	51.8	1.8	3.4	0.05	<0.01
Smallmouth bass	30	65.0	1.6	0.4	0.27	0.58
Largemouth bass	132	75.2	0.5	0.2	0.10	0.25
White crappie	90	72.3	0.2	0.2	0.45	0.37
Yellow perch	48	62.1	0.4	0.1	0.50	0.70
Walleye	35	40.8	0.6	0.2	0.57	0.76

[a]Note separate regressions for rising and falling field temperatures in Table 1.

TABLE 3. Covariance analysis of avoidance: acclimation temperature regressions for species within families for Muddy Run–Conowingo (Pennsylvania) fishes. Differences within each family among intercepts (a) and slopes (b) of species regressions were rejected for $P > 0.01$ or when the amount of variance explained was small.

			% of total sum of squares			Probability	
Family	No. of species	N	Acclimation temperature (X)	Intercept (a)	Slope (b)	Intercept (a)	Slope (b)
Cyprinidae	4	268	72	1.0	0.5	0.06	0.22
Ictaluridae	2	108	63	1.0	0.5	0.98	0.80
Centrarchidae	7	459	64	7	3	<0.01	<0.01
Centrarchidae[a]	6	369	64	3	3	<0.01	<0.01
Percidae	2	83	52	1	1	0.25	0.28

[a]Excluding white crappie data.

effects significantly detectable even at $P = 0.01$ (Mathur and Silver 1980), we wanted to be certain that a given source of variation made an important contribution to the total variance (R^2) before including that variable in the model. The 99% confidence level allowed for meaningful evaluation of R^2 values and was selected to provide a suitable compromise between type I and type II errors and added conservatism. Using $P = 0.01$ to include a term in the model causes the model to be as simple as possible and makes it less likely that the model will adequately predict some data. Using $\alpha = 0.10$ to flag such data points via confidence intervals makes it more likely than when using $P = 0.05$ or 0.01 that a given data point will be "flagged" as an outlier, i.e. that the model failed to predict that observation. The smaller the α "to include" and the larger the α for "flagging" the more likely the model is to fail to predict any given observation. Both choices are therefore biased toward conservatism.

The equations obtained for each species and for periods of rising and falling field temperatures were validated by a random selection technique using the Statistical Analysis System (Barr et al. 1976). These validation procedures are described in Mathur et al. (1981).

TEST OF ASSUMPTIONS

We tested for violations of assumptions for parametric statistical techniques as described in Mathur et al. (1981). We examined (1) overall residuals (observed minus predicted values) for normality, (2) residuals versus fitted values, and (3) residuals versus independent variables. These analyses generally supported adequacy of the fitted model, and the statistical assumptions did not reveal serious inadequacy. Moderate departures from normality have little or no effect on conclusions reached through calculation of the F-statistic (Scheffe 1959).

TABLE 4. Regression statistics (avoidance temperature versus acclimation temperature) for prediction of avoidance temperatures of fish taxa. Analyses from Muddy Run–Conowingo (Pennsylvania) were considered separately and in combination with published data (references are in Table 5). R^2 = coefficient of determination. Standard errors of estimates (SE) given here were used for setting confidence limits in Fig. 2.

Taxon	No. of tests	No. of species	R^2	SE	Intercept (a)	Slope (b)
			Muddy Run–Conowingo data			
Cyprinidae	268	4	0.73	3.35	14.72	0.65
Ictaluridae	108	2	0.64	4.55	14.36	0.73
Centrarchidae	369	6	0.64	3.49	20.14	0.53
White crappie	90	1	0.72	3.08	15.23	0.62
Percidae	83	2	0.52	3.16	19.74	0.46
			All data			
Cyprinidae	354	9	0.73	3.26	14.59	0.64
Ictaluridae	120	2	0.63	4.51	14.87	0.72
Centrarchidae	424	8	0.66	3.34	20.02	0.53
White crappie	90	1	0.72	3.08	15.23	0.62
Percidae	91	2	0.55	3.19	18.99	0.49

TABLE 5. Data sources used for analysis of geographic and familial variations in avoidance temperatures of freshwater fishes.

Species	Studies	Locality	Range of acclimation temperature (°C)
Cyprinidae			
Stoneroller, *Campostoma anomolum*	Cherry et al. (1975, 1977)	Virginia	6.0–30.0
Swallowtail shiner, *Notropis procne*	This study	Pennsylvania	0.0–28.9
Rosyface shiner, *Notropis rubellus*	Cherry et al. (1975, 1977)	Virginia	6.0–33.0
Spottail shiner, *Notropis hudsonius*	This study	Pennsylvania	13.9–26.7
Spotfin shiner, *Notropis spilopterus*	Cherry et al. (1975, 1977)	Virginia	6.0–36.0
	This study	Pennsylvania	0.0–28.3
Telescope shiner, *Notropis telescopus*	Cherry et al. (1977)	Virginia	12.0–27.0
Bluntnose minnow, *Pimephales notatus*	Cherry et al. (1975, 1977)	Virginia	6.0–30.0
	This study	Pennsylvania	0.5–27.2
Fathead minnow, *Pimephales promelas*	Cherry et al. (1975, 1977)	Virginia	12.0–30.0
Bluehead chub, *Nocomis leptocephalus*	Cherry et al. (1975)	Virginia	12.0–24.0
Ictaluridae			
Brown bullhead, *Ictalurus nebulosus*	This study	Pennsylvania	13.9–26.7
Channel catfish, *Ictalurus punctatus*	Cherry et al. (1975)	Virginia	6.0–30.0
	This study	Pennsylvania	0.6–32.2
Centrarchidae			
Rock bass, *Ambloplites rupestris*	Cherry et al. (1977)	Virginia	18.0–36.0
Pumpkinseed, *Lepomis gibbosus*	This study	Pennsylvania	17.0–32.2
Green sunfish, *Lepomis cyanellus*	Cherry et al. (1975)	Virginia	6.0–30.0
	This study	Pennsylvania	3.3–26.1
Bluegill, *Lepomis macrochirus*	Cherry et al. (1975, 1977)	Virginia	12.0–36.0
Smallmouth bass, *Micropterus dolomieui*	Cherry et al. (1975, 1977)	Virginia	15.0–33.0
	This study	Pennsylvania	10.0–32.2
Spotted bass, *Micropterus punctulatus*	Cherry et al. (1975, 1977)	Virginia	6.0–36.0
Largemouth bass, *Micropterus salmoides*	This study	Pennsylvania	1.1–33.9
Percidae			
Yellow perch, *Perca flavescens*	Cherry et al. (1977)	Virginia	15.0–24.0
	This study	Pennsylvania	5.0–29.4
Walleye, *Stizostedion vitreum*	This study	Pennsylvania	0.0–27.8

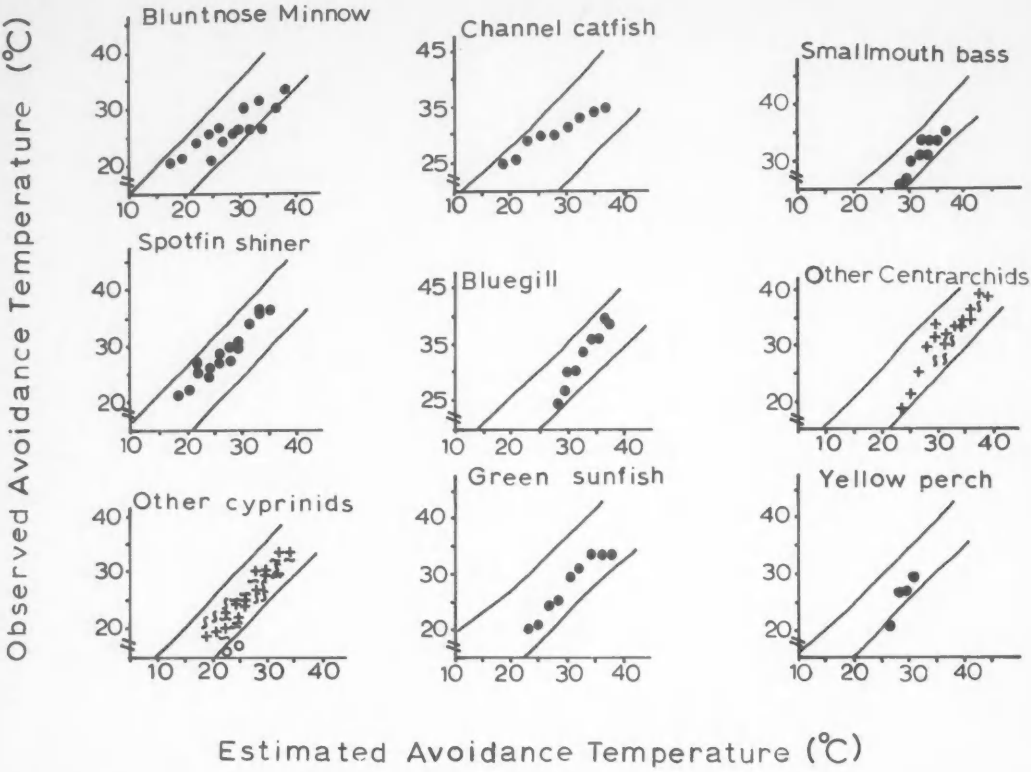

FIG. 1. Comparison of the observed and predicted avoidance temperature of fishes and 90% confidence intervals. Acclimation temperature is the independent variable in the underlying regressions. Confidence intervals are derived from Muddy Run–Conowingo (Pennsylvania) data, which are not shown. Equations given in Tables 3 and 6 were used to predict avoidance temperatures of fishes tested by others. Cyprinids: ●, bluntnose minnow and spotfin shiner (Cherry et al. 1975, 1977); ○, bluehead chub (Cherry et al. 1975) and fathead minnow (Cherry et al. 1975, 1977); ~, rosyface shiner (Cherry et al. 1975, 1977); ∫, stoneroller minnow (Cherry et al. 1975, 1977); +, telescope shiner (Cherry et al. 1977). Ictalurids: ●, channel catfish (Cherry et al. 1975). Centrarchids: ●, bluegill, smallmouth bass (Cherry et al. 1975, 1977) and green sunfish (Cherry et al. 1975); +, spotted bass (Cherry et al. 1975, 1977); ×, rock bass (Cherry et al. 1977). Percidae: ●, yellow perch (Cherry et al. 1977).

Results

PENNSYLVANIA DATA

Acclimation temperature had a direct positive effect upon avoidance temperatures of species tested (Table 1). Fishes avoided temperatures higher than their acclimation temperatures. The differences between avoidance and acclimation temperatures decreased as acclimation temperatures increased, a common feature of such studies. Avoidance temperatures ranged from 7.2 to 39.4°C for the acclimation temperatures tested (0.0–33.9°C). Temperature differentials ranged from 1.1 to 26.7°C. For example, the largemouth bass, *Micropterus salmoides*, acclimated at 30.0°C avoided 36.1°C, a differential of 6.1°, but when acclimated at 5.0°C avoided 20.6°, a differential of 15.6°.

Correlation coefficients for acclimation and avoidance temperature relationships for each species were significant ($P < 0.001$), but some variation was observed (Table 1). Acclimation temperature explained 41% (walleye, *Stizostedion vitreum*, and brown bullhead, *Ictalurus nebulosus*) to 88% (spottail shiner, *Notropis hudsonius*) of the total variance (R^2) in the avoidance temperatures of fishes. The low correlations for some species resulted from a variable response associated with acclimation temperatures of 15.6°C and lower.

Other variables (light, pH, acclimation time, and length of fish) were not significant ($P > 0.01$) in predicting the avoidance temperatures. Since a large amount of the variance remained unexplained for certain species, other unknown factors not considered or measured in the present study must also affect avoidance temperatures.

No significant differences ($P > 0.01$) in temperatures

TABLE 6. Covariance analysis of new and published avoidance temperature of fishes. Sums of squares due to each source of variation, expressed as percent of total sum of squares, are given in parentheses. "Author" refers to the source of published data and regression intercepts (test for homogeneity of intercepts). "Acclimation * author" refers to regression slopes (test for homogeneity of slopes).

Source of variation	df	Sum of squares	Mean square	*F*	Probability
		Bluntnose minnow			
Acclimation temperature	1	2378 (69)	2378.0	179.1	0.01
Author	1	21 (0.6)	21.0	1.6	0.20
Acclimation temperature * author	1	30 (1)	30.0	2.3	0.13
Error	76	1009	13.3		
Total	79	3438			
		Spotfin shiner			
Acclimation temperature	1	4422 (70)	4422.0	370.8	0.01
Author	1	33 (0.5)	33.0	2.8	0.10
Acclimation temperature * author	1	3 (<0.1)	3.0	0.3	0.60
Error	154	1836	12.0		
Total	157	6294			
		Channel catfish			
Acclimation temperature	1	3621 (64)	3621.0	164.3	0.01
Author	1	71 (1)	71.0	3.2	0.08
Acclimation temperature * author	1	52 (1)	52.0	2.4	0.13
Error	87	1917	22.0		
Total	90	5661			
		Bluegill			
Acclimation temperature	1	1247 (57)	1247.0	135.4	0.01
Author	1	1 (<0.1)	1.0	0.2	0.70
Acclimation temperature * author	1	27 (1)	27.0	2.9	0.09
Error	99	912	9.2		
Total	102	2187			
		Green sunfish			
Acclimation temperature	1	672 (69)	672.0	62.3	0.01
Author	1	46 (5)	46.0	4.3	0.05
Acclimation temperature * author	1	1 (<0.1)	1.0	1.0	0.83
Error	24	259	10.8		
Total	27	978			
		Smallmouth bass			
Acclimation temperature	1	515 (71)	515.0	114.1	0.01
Author	1	22 (3)	22.0	4.9	0.03
Acclimation temperature * author	1	1 (<0.5)	1.0	0.1	0.72
Error	42	189	4.5		
Total	45	727			
		Yellow perch			
Acclimation temperature	1	805 (69)	805.0	127.5	0.01
Author	2	19 (2)	9.5	1.5	0.24
Acclimation temperature * author	2	30 (3)	15.0	2.3	0.11
Error	50	315	6.3		
Total	55	1168			

avoided by fishes were evident, except for bluntnose minnow, *Pimephales notatus*, during seasons of rising and falling field temperatures (Table 2). In the case of bluntnose minnow the variation due to differences among seasons was small (<5%) and of low predictability. At a common acclimation temperature the bluntnose minnow avoided lower temperatures

TABLE 7. Covariance analysis of geographical variations in species within a family for avoidance temperatures of fishes. Sums of squares due to each source of variation, expressed as percent of the total sum of squares, are given in parentheses. "Species" refers to regression intercepts and "acclimation * species" to regression slopes. Significance of variance contributions was rejected for $P > 0.01$ or when the amount of information explained was small.

Source of variation	df	Sum of squares	Mean square	F	Probability
		Cyprinidae			
Acclimation temperature	1	10 049 (73)	10 049.0	1004.7	0.01
Species	8	335 (2)	41.9	4.2	0.01
Acclimation temperature * species	8	44 (0.3)	5.5	0.6	0.82
Error	336	3 360	10.0		
Total	353	13 788			
		Ictaluridae			
Acclimation temperature	1	4 152 (63)	4 152.0	201.2	0.01
Species	1	1 (<0.1)	1.0	0.1	0.81
Acclimation temperature * species	1	1 (<0.1)	1.0	0.1	0.89
Error	116	2 394	20.6		
Total	119	6 548			
		Centrarchidae			
Acclimation temperature	1	9 192 (66)	9 192.0	967.4	0.01
Species	7	381 (3)	54.4	5.7	0.01
Acclimation temperature * species	7	458 (3)	65.4	6.9	0.01
Error	408	3 877	9.5		
Total	424	13 908			
		Percidae			
Acclimation temperature	1	1 109 (55)	1 109.0	114.9	0.01
Species	1	32 (2)	32.0	3.3	0.07
Acclimation temperature * species	1	31 (2)	31.0	3.2	0.08
Error	87	840	9.7		
Total	90	2 012			

during the period of falling field temperatures. Both the slopes and elevations were similar in the two periods for other species. Thus, if acclimation temperatures of a species were the same in the two periods, the avoided temperatures would be similar.

There were few differences in avoidance temperature responses among species within a family (Table 3). The cyprinids, ictalurids, and percids did not differ significantly among themselves nor did six centrarchids. The removal of white crappie (*Pomoxis annularis*) data from the analysis reduced the variance contribution of species from 7 to 3%, a significant reduction. Consequently, two sets of validated regression equations were developed, one for white crappie and one for the other six centrarchids (Table 4). White crappie showed a similar response in its acute preferences (Mathur et al. 1981).

PUBLISHED DATA

Only the data of those studies in which both the acclimation and corresponding avoidance temperatures were available, either in a tabular or graphical form, were used. No published data were ignored or deleted in our analysis. If only a range of temperatures was given by the author(s) the data could not be included in our statistical analysis. In contrast with the acute preference studies, data on avoidance temperatures of fishes were available for fewer species and from more limited sources.

We first examined confidence intervals to assess populational outliers within species. We assumed that the confidence intervals generated from our study as described earlier represent the sampling variation. Observed values that significantly differ from the predicted values should fall outside the specified interval and may be attributed to geographical or species effects. Because of a relatively small sample size for each species this method of flagging outliers is relatively efficient.

Published acclimation temperatures for each species (Table 5) were substituted into respective equations (Table 1) and avoidance temperatures were estimated. For species not tested in our laboratory but tested by others, family regressions (Table 4) were used for prediction. These estimated temperatures were compared with those observed by the other investigators. The regression models for the families predicted satisfactorily avoidance temperatures of species not tested in our laboratory (Fig. 1). The avoidance temperatures of bluehead chub, *Nocomis leptocephalus* (a species not

tested in our laboratory), predicted from our model were greater than those observed by Cherry et al. (1975). However, the avoidance temperatures reported by Cherry et al. (1975) were only 1−4.0° higher than the five acclimation temperatures they tested (12.0−21.0°C). The bluehead chub was categorized as a "coldwater" species, which may explain the lack of agreement.

We determined via covariance analysis whether the means of avoidance temperatures (adjusted for acclimation temperature) were significantly different ($P < 0.01$). We compared avoidance temperatures of species individually and grouped by family. The contribution of "author" to the total variance using acclimation temperature as a covariate was examined in testing geographical differences for a species. We analyzed the variance contribution of "species" in testing differences among species within a family. The analysis included all cases where authors had provided at least three discrete matched pairs of avoidance−acclimation temperatures.

Covariance analyses generally corroborated the conclusions based on confidence intervals (Tables 6 and 7). In conventional terms this analysis can be viewed as testing whether a single intercept and slope is satisfactory for the several acclimation temperatures and authors (geographical areas). This test is provided by the sums of squares for "author" and "author * acclimation temperature" (Barr et al. 1976). This is equivalent to the test suggested by Kempthorne (1952) in which the two degrees of freedom are combined. In either case, differences among slopes and intercepts account for only 1.6% (except for bluntnose minnow) of the total variance, and neither source approaches statistical significance or practical value. The contribution of variance due to author was not more than 5% of the total variation in comparisons of individual species. In comparisons of species within a family, the sum of squares due to differences in species contributed less than 4% of the total sum of squares, and the variance due to this source was not significant ($P > 0.01$). The acclimation temperature was the most important source of variation in both analyses, and sums of squares due to this source accounted for up to 75% of the total sum of squares. The statistical similarity of avoidance responses provided a basis for pooling available data by family (Table 4; Fig. 2).

Discussion

Strong evidence of similarities rather than differences between populations and among species within a family was demonstrated. We recognize that the present analysis includes only a few studies and each with a few observations per species. However, we are optimistic about the potential applicability of the generalities emerging from this study because the same warmwater fishes exhibited a remarkable degree of similarity in laboratory acute temperature preferences (Mathur et al. 1981). As in the case of acute preference responses, some statistical differences that occurred were not

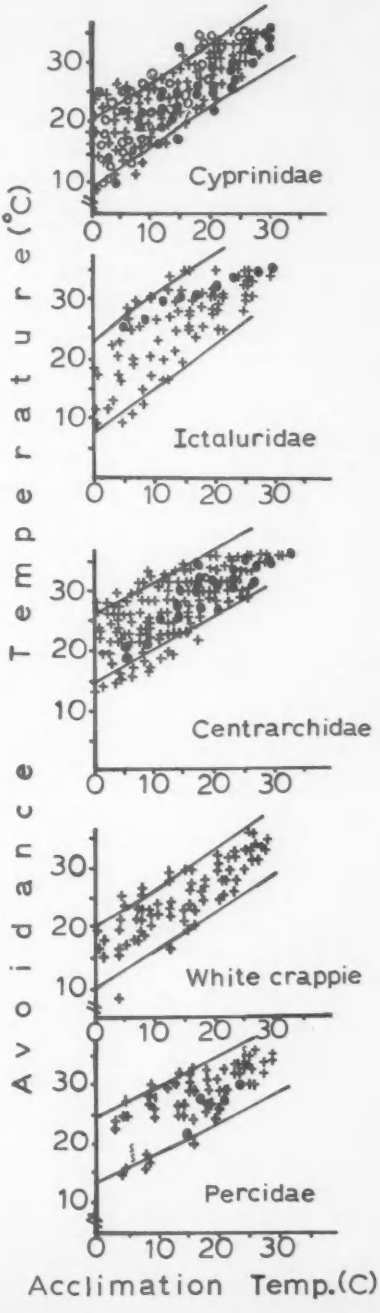

FIG. 2. Plot of the published data on the relationship between avoidance temperature and acclimation temperatures of fishes within a family. The 90% confidence intervals of the individual predicted values are shown. ●, Cherry et al. (1975, 1977); +, Muddy Run−Conowingo.

considered important from a practical or biological standpoint. The amount of additional variance explained, or increase in precision, by including numerous variables in the model was small compared with the effect of acclimation temperature. The generalities emerging from the statistical analysis of the available avoidance data sets should lower the research costs of thermal impact assessments where specific data are lacking or difficult to obtain in a short time using current methodologies.

The significant effect of acclimation temperature coupled with the high variability often associated with these types of tests, in our view, precludes the detection of geographical differences in responses of most species from the available laboratory data sets. Consequently, when geographically isolated populations of the same species, even with different acclimation regimes, are tested in a laboratory, thermal responses tend to be indistinguishable.

A large amount of subjectivity is injected into analysis of thermal fish behavior, particularly when literature comparisons are made. The type of statistical analysis offered here, by accounting for random variation, can isolate true differences or similarities among or within studies. The analysis provides a basis for predicting avoidance temperatures of fishes within the specified variation. This approach can generate insights in many cases where available data may seem insufficient or inconsistent and can help focus future research needs (Mathur et al. 1981, 1982).

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A New Approach to Estimating Populations by the Removal Method

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SCHNUTE, J. 1983. A new approach to estimating populations by the removal method. Can. J. Fish. Aquat. Sci. 40: 2153–2169.

This paper presents a new approach to the use of removal data in estimating the size of a population of fish or other animals. The theory admits a variety of assumptions on how catchability varies among fishings including the assumption of constant catchability, which underlies most previous work. The methods here hinge on maximum likelihood estimation, and they can be used both to decide objectively if the data justify rejecting constant catchability and to determine confidence intervals for the parameters. The work includes a new method of assigning confidence to the population estimate and points out problems with methods currently available in the literature, even in the case of constant catchability. The theory is applied both to data in historical literature and to more recent data from streams in New Brunswick, Canada. These examples demonstrate that the assumption of constant catchability can frequently lead to serious errors in data interpretation. In some cases, the conclusion that the population size is well known may be blatantly false, and reasonable estimates may be impossible without further data.

SCHNUTE, J. 1983. A new approach to estimating populations by the removal method. Can. J. Fish. Aquat. Sci. 40: 2153–2169.

Le présent article contient une approche nouvelle de l'utilisation des données sur la récolte dans l'estimation de l'effectif d'une population de poissons ou d'autres animaux. Diverses hypothèses sont admises quant à la manière dont le potentiel de capture varie d'une pêche à l'autre, y compris celle d'un potentiel de capture uniforme que présupposent la plupart des travaux antérieurs. Les méthodes exposées ici sont fondées sur l'estimation de probabilité maximale, et peuvent être utilisées à la fois pour décider objectivement si les données justifient le rejet d'un potentiel de capture uniforme et déterminer, pour les paramètres, des intervalles de confiance. Nous décrivons une nouvelle méthode pour définir des limites de confiance d'une estimation de population et attirons l'attention sur les problèmes associés aux méthodes décrites dans la littérature courante, même dans le cas d'un potentiel de capture uniforme. Nous appliquons la théorie aux données contenues dans des travaux publiés dans le passé, ainsi qu'à des données récentes sur des cours d'eau du Nouveau-Brunswick, au Canada. Ces exemples démontrent que l'hypothèse d'un potentiel de capture uniforme peut fréquemment être la cause de sérieuses erreurs d'interprétation des données. Dans certains cas, il se peut que la conclusion que l'effectif d'une population est bien connu soit tout à fait fausse et que, sans d'autres données, il peut être impossible d'arriver à des estimations raisonnables.

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CONCEPTUALLY, the removal method of estimating an animal population is perhaps the simplest available. The idea is to capture a fixed fraction of the population on several occasions and use the resulting trend to estimate the starting point. For instance, suppose that a pond is fished three times with fixed effort and that the resulting catches (removed each time) are 90, 60, and 40. In this sequence, each catch is reduced by 1/3 from the previous one. If the catch is proportional to the available population, then it is reasonable to conclude that the population is reduced by 1/3 on each fishing. Consequently, the first catch of 90 represents 1/3 of the starting population, which must be 270. The following scenario perfectly explains the data:

Population	Catch	Capture probability
270	90	1/3
180	60	1/3
120	40	1/3

The main assumptions here are that the probability of capture is constant on each occasion and that the population is closed; i.e. there is no immigration, emigration, birth, or natural mortality.

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The method has been used frequently in fisheries applications, particularly in small restricted areas such as ponds, small lakes, and streams. A variation of the method allows for different effort on each capture, and this is sometimes applied in commercial marine fisheries. Of course, the data normally do not follow a perfect progression as in the example above, and it is necessary to develop statistical methods for estimating the initial population and finding the probable error of the estimate. Major papers on the subject include those by Leslie and Davis (1939), DeLury (1947), Hayne (1949), Moran (1951), Zippin (1956), Seber and Le Cren (1967), and Otis et al. (1978). For a historical review, see Ricker (1975, p. 150ff). These papers progress toward greater degrees of statistical rigor; for instance, Moran (1951) first formulates the statistical likelihood function for the problem and obtains approximate formulae for the maximum likelihood estimate. Except for the monograph by Otis et al. (1978), all the papers cited above were written before computing power was as easily available as it is today, and for that reason they typically present graphical and approximate methods for dealing with awkward numerical calculations.

The main limitation of most earlier work is the underlying assumption of constant capture probability. Writers recognized the problem but did not seriously address it. For example, Moran (1951, p. 310) noted that "if the animals become trap-shy as a result of trapping, the theory breaks down and the true population size is then probably greater than that estimated. There is evidence that trap-shyness does occur in some animals, and in the present data there is perhaps some slight indication of it in the rather large drop in the second catch relative to the first." Cross and Scott (1975) in their own data noticed a very large drop in the second catch relative to the first, and they observed that if one applies the simplest regression method to their data, it leads to the untenable conclusion that the initial population must have been less than the total catch from all fishings. They also devised an ad hoc method for handling the problem of increased resistance to capture after the first fishing. Unfortunately, their method is provably inconsistent with their own assumption that the first capture probability is distinct from that on subsequent fishings.

Otis et al. (1978) examined in considerable generality the problem of nonconstant capture probabilities, and this paper explores in depth some of their ideas. The theory here admits arbitrary structure in the capture probability as the number of fishings increases. It includes the classical case of constant catchability, as well as the slightly more general case of a distinct first catchability with a different constant catchability thereafter. It also includes the possibility of a steadily decreasing (or increasing) catchability, along with statistical tests to decide which of these various possibilities is most appropriate. The estimation methods described here are exact, given the statistical theory. Approximations and graphical methods can be completely avoided, although graphs prove useful in assessing the results. Even though the calculations are somewhat elaborate, they can easily be programmed on a small computer. For example, all analysis reported here was performed on an Apple II microcomputer.

This paper also examines methods of determining confidence limits for estimates of model parameters. It identifies several theoretical and practical problems with earlier methods of finding these limits, particularly for the estimated initial population size. A method that avoids these problems is described and applied. In cases where nonconstant catchability is statistically justified, the confidence in the population estimate is sometimes improved and sometimes worsened. In the worst cases, no upper limit on population size can be determined; i.e. it is impossible to reject statistically any presumed population larger than the total catch. The only reasonable recourse in such cases is to continue fishing. A "rule of thumb," simple enough to apply while still at the fishing site, is given for deciding whether or not to continue fishing.

The methods are illustrated with data collected in New Brunswick, Canada, on brook trout (*Salvelinus fontinalis*) taken from Hayden and Upper Narrows Mountain brooks as a part of the Nashwaak Experimental Watersheds Project, 1974–76. From a collection of 99 available samples, 16 are selected by the simple rule that the total catch should be at least 40 fish. The null hypothesis of constant capture probability can be rejected with 95% confidence in 8 (or 50%) of these 16 samples in favor of the hypothesis of a distinct initial probability. In all 8 such samples, this change in hypothesis affects both the population estimate and its confidence limits. In some cases, the changes are so radical as to alter totally the conclusions that can be drawn from the sample.

This paper points out limitations of almost any method ever proposed to analyze removal data, even in the case of constant capture probability. The final section compares the methods used here with those of earlier papers and speculates on possible directions for future work.

1. Deterministic Models

To introduce the models considered here, it is convenient first to ignore all probabilistic aspects of the removal procedure. Concepts and notation developed in this section make it easier to define the complete statistical model presented in section 2. As an aid to the reader, all notation in the paper is summarized in Appendix A.

Let N be the initial population from which k catches C_i, $i = 1,\ldots,k$, are taken. The total catch up to and including the ith catch is

$$(1.1) \quad T_i = \sum_{j=1}^{i} C_j, \quad i = 1,\ldots,k.$$

For convenience, also define $T_0 = 0$. The proportion of remaining fish caught (the "capture fraction") on the ith catch is then

$$(1.2) \quad q_i = C_i/(N - T_{i-1}), \quad i = 1,\ldots,k$$

and the proportion escaped (the "escapement fraction") is

$$(1.3) \quad p_i = 1 - q_i, \quad i = 1,\ldots,k.$$

Equations (1.1)–(1.2) show that the $k + 1$ quantities $N,C_1,\ldots,C_k$ determine the q's. Similarly, the quantities $N,q_1,\ldots,q_k$ determine the C's by the formula

$$(1.4)\quad C_i = q_i N \prod_{j=1}^{i-1} (1 - q_j), \quad i = 1,\ldots,k$$

where the product in (1.4) is defined to be 1 when $i = 1$.

In the discussion above, (1.1) and (1.3) can be considered definitions. Given (1.1), it is possible to derive (1.4) from (1.2) and vice versa. This result is derived in Appendix B. Regardless of which equation (1.2) or (1.4) is taken as fundamental, there are so far $k + 1$ quantities that define the problem: either N and the C's or N and the q's. For the problem to make sense biologically, these quantities must be constrained to be realistic values, namely,

$$(1.5)\quad C_i \geq 0, \quad N \geq T_k$$

or

$$(1.6)\quad 0 \leq q_i \leq 1, \quad N \geq 0.$$

Again, these constraints are equivalent; i.e. each implies the other, given (1.1) and either (1.2) or (1.4).

In practice, there are only k observed catches, so the number of parameters must be reduced from $k + 1$ if the parameters are to be found from the observations. This can be done by assuming structure in the capture fractions. The classical assumption (call it "model 1") is that they are constant:

$$(1.7)\quad q_i = q_1, \quad i = 1,\ldots,k.$$

Model 1, then, has two parameters: N and q_1. Equation (1.7) describes the q's in terms of these parameters (where, in fact, the q's are independent of N) and the C's are given by

$$(1.8)\quad C_i = q_1 (1 - q_1)^{i-1} N, \quad i = 1,\ldots,k.$$

This result follows from (1.4) and (1.7). Alternatively, model 1 can be specified in terms of the two parameters N and C_1; in this case the q's and C's are given by

$$(1.9)\quad q_i = C_1/N, \quad i = 1,\ldots,k$$

and

$$(1.10)\quad C_i = C_1 [(N - C_1)/N]^{i-1}, \quad i = 1,\ldots,k.$$

Define "model 2" similarly to model 1, except allow the first capture fraction to differ from the others; i.e. assume that

$$(1.11)\quad q_i = \begin{cases} q_1, & i = 1 \\ q, & i = 2,\ldots,k. \end{cases}$$

Thus, model 2 is specified by the three parameters N, q_1, and q and it reduces to model 1 when q equals q_1. Equation (1.11) gives the q's for model 2, just as (1.7)gives them for model 1. Similarly

$$(1.12)\quad C_i = \begin{cases} q_1 N, & i = 1 \\ q (1 - q_1) (1 - q)^{i-2} N, & i = 2,\ldots,k \end{cases}$$

is the counterpart of (1.8), now based on (1.4) and (1.11), which gives the C's for model 2. Alternatively, model 2 can be specified by the three parameters N, C_1, and C_2. The q's and C's are then given by

$$(1.13)\quad q_i = \begin{cases} C_1/N, & i = 1 \\ C_2/(N - C_1), & i = 2,\ldots,k \end{cases}$$

$$(1.14)\quad C_i = \begin{cases} C_1, & i = 1 \\ C_2 [(N - C_1 - C_2)/(N - C_1)]^{i-2}, & i = 2,\ldots,k. \end{cases}$$

It may happen that the initial capture fraction q_1 does not change suddenly to the final fraction q in the second fishing, but instead does so gradually. Define "model 3" by the structure

$$(1.15)\quad q_i = q_1 + (q - q_1) (1 - a^{i-1}), \quad i = 1,\ldots,k$$

where a is constant with $0 \leq a \leq 1$. Notice that the right-hand side of (1.15) reduces to q_1 when $i = 1$ and (for $a < 1$) tends to q as i becomes large. Model 3 contains models 1 and 2 as special cases. When $a = 1$, (1.15) becomes (1.7); i.e. model 3 becomes model 1. Similarly, when $a = 0$ (or, more precisely, as a tends to 0), (1.15) becomes (1.11), and model 3 reduces to model 2. The three equations (1.7), (1.11), and (1.15) thus form a progression. Similarly, model 3 has a counterpart to (1.8) and (1.12) that expresses the catches C_i in terms of N and the q's, but that equation is awkward to state. It results simply from combining (1.4) and (1.15).

Model 3 has four parameters: N, q_1, q, and a. As described above, these determine all the q's and C's. The model can also be specified by the four parameters N, C_1, C_2, and C_3. In terms of these, it turns out that

$$(1.16)\quad q_i = C_i/(N - T_{i-1}), \quad i = 1,2,3$$

$$(1.17)\quad q = (q^2 - q_1 q_3)/(2q_2 - q_1 - q_3)$$

$$(1.18)\quad a = (q_3 - q_2)/(q_2 - q_1).$$

Notice that (1.16)−(1.18) allow one to calculate, successively, q_1, q_2, q_3, q, and a from N and the first three C's and thus to convert from the second parameter set to the first.

Models 2 and 3 extend the structure of capture fractions beyond the simple classical case of model 1. They should not, however, be regarded as the only possible or reasonable such extensions. For example, it may be that fishings are performed with alternating gear types, so that the experimenter might wish to associate one capture fraction with odd-numbered fishings and a second fraction with even-numbered fishings. The likelihood estimation methods described in the next section can be applied to any such model.

2. Maximum Likelihood Estimation

The previous section describes ways to model removal data based on a small set of parameters. Let $\mathbf{Z}$ denote such a parameter set; e.g.

$$\mathbf{Z} = (N, q_1) \text{ in model 1}$$

$\mathbf{Z} = (N,q_1,q)$ in model 2

$\mathbf{Z} = (N,q_1,q,a)$ in model 3.

In general, $\mathbf{Z}$ is a vector of m components

$$(2.1) \quad \mathbf{Z} = (z_1,\ldots,z_m)$$

where, e.g., m is 2, 3, or 4 in models 1, 2, or 3, respectively. Typically, the first parameter z_1 is the initial population N, and the remaining parameters define the capture fractions. There may be several equivalent sets of parameters; for instance, as shown in section 1, either (N,q_1) or (N,C_1) can be used to define model 1.

Once $\mathbf{Z}$ is known, not only the q's, but also the C's are determined. These C's are "predicted" catches for the model when the corresponding q's are interpreted as capture "probabilities" rather than capture fractions as in the deterministic description. The observed "catches" $\hat{C}_1,\ldots,\hat{C}_k$, obtained by experiment, naturally do not agree exactly with the predicted catches, and statistical analysis of the data is based on investigating this disagreement. Notice that the C's depend only on the model parameters $\mathbf{Z}$. They correspond to predictions prior to making any observations, and by definition are computed from (1.4), or equivalent expressions such as (1.8), (1.10), (1.12), or (1.14). Similarly, the T's (predicted cumulative catches) are determined entirely by $\mathbf{Z}$ from (1.1).

In analogy with (1.1), define

$$(2.2) \quad \hat{T}_i = \sum_{j=1}^{i} \hat{C}_j, \quad i = 1,\ldots,k$$

i.e. the sum of the first i observed catches. (As before, define $\hat{T}_0 = 0$.) On the ith fishing, each of the remaining $N - \hat{T}_{i-1}$ fish is exposed to capture with probability q_i. Based on a binomial model of fish capture, the probability that C_i fish are caught and the remaining $N - \hat{T}_i$ fish escape is

$$(2.3) \quad \text{pr}\{\hat{C}_i|N,\hat{T}_{i-1},q_i\} = \binom{N - \hat{T}_{i-1}}{\hat{C}_i}(1 - q_i)^{N-\hat{T}_i}q_i^{\hat{C}_i}.$$

The probability of seeing the complete set of k observed catches (the "likelihood") is the product of these probabilities:

$$(2.4) \quad L(\hat{C}_1,\ldots,\hat{C}_k|\mathbf{Z}) = \frac{N!}{(N - \hat{T}_k)!}\prod_{i=1}^{k}\frac{q_i^{\hat{C}_i}(1 - q_i)^{N-\hat{T}_i}}{\hat{C}_i!}.$$

When writing the product (2.4), the binomial coefficients in (2.3) have been replaced by their values in terms of factorials:

$$\binom{N - \hat{T}_{i-1}}{\hat{C}_i} = \frac{(N - \hat{T}_{i-1})!}{(N - \hat{T}_i)!\,\hat{C}_i!}, \quad i = 1,\ldots,k.$$

Most of the factorials $(N - \hat{T}_i)!$ cancel in the product.

The binomial model used here involves a probability associated with the observed catch $\hat{C}_i$, given the available population $N - \hat{T}_{i-1}$ determined from N and the previous catches. The model has the property (Fisz 1963, p. 66, eq. (3.1.6)) that the expected value of $\hat{C}_i$ is

$$(2.5) \quad E\{\hat{C}_i|N,\hat{T}_{i-1}\} = q_i(N - \hat{T}_{i-1}).$$

This expected value is different, in general, from the prediction $C_i(\mathbf{Z})$ prior to making any observations.

In (2.4) notice that the parameter vector $\mathbf{Z}$ determines N and the q's; in this way L depends on $\mathbf{Z}$. The value of $\mathbf{Z}$ that maximizes L is called the "maximum likelihood estimate"; it corresponds to the value that makes the data look most probable. However, for numerical reasons the function L is not the most convenient one to maximize; it is better to use the negative logarithm of L, possibly adjusted by a constant. It turns out (Appendix C) that

$$(2.6) \quad -\log L(\mathbf{Z}) = G(\mathbf{Z}) + H(\mathbf{Z}) + K$$

where

$$G(\mathbf{Z}) = N\log(N) - \hat{T}_k\log(\hat{T}_k) - (N - \hat{T}_k)\log(N - T_k) - \log\binom{N}{\hat{T}_k}$$

$$H(\mathbf{Z}) = \sum_{i=1}^{k}\hat{C}_i\log(\hat{C}_i/C_i)$$

$$K = \sum_{i=1}^{k}[\log(\hat{C}_i!) - \hat{C}_i\log(\hat{C}_i)] - \log(\hat{T}_k!) + \hat{T}_k\log(\hat{T}_k).$$

Since $\mathbf{Z}$ determines N and the C's and since the C's determine T_k, it follows that G and H both depend on $\mathbf{Z}$; however, K does not, since it involves only the observations. Consequently, the function

$$(2.7) \quad F(\mathbf{Z}) = G(\mathbf{Z}) + H(\mathbf{Z})$$

can be used in place of $L(\mathbf{Z})$ as a criterion for estimating the parameter vector $\mathbf{Z}$.

As (2.7) shows, the function F has two parts. The second, H, is related to the closeness between observed and predicted catches; notice that $H = 0$ when $C_i = \hat{C}_i$. The first part, G, is related to the closeness between observed and predicted values of total catch; notice that $G = 0$ when $N = T_k = \hat{T}_k$. (This involves the facts that $x\log x$ tends to 0 as x tends to 0 and that the binomial coefficient $\binom{x}{x}$ is 1.) Another significance to G is that it relates two measures of the fish population left after all fishings, $N - T_k$ and $N - \hat{T}_k$. The function F has several important properties. These are listed below.

PROPERTY 1

The maximum likelihood estimate for $\mathbf{Z}$ is the same as the value of $\mathbf{Z}$ that minimizes F.

PROPERTY 2

If $N \geq \hat{T}_k$, then $F \geq 0$. Furthermore, $F = 0$ if and only if both

$$(2.8) \quad C_i = \hat{C}_i, \quad i = 1,\ldots,k$$

and

$$(2.9) \quad N = \hat{T}_k.$$

PROPERTY 3

Let F_1' be the minimum value of F for model 1 and let F_2' be the minimum for model 2. Suppose that model 1 is valid. Then $2(F_1' - F_2')$ is approximately χ^2 distributed with 1 *df*. More generally, if F' is the minimum F for one model and F'' is the minimum F for an extended model with n additional parameters, then $2(F'-F'')$ is approximately χ^2 distributed with n *df* when the first model is true.

PROPERTY 4

Suppose that

$$(2.10) \quad \hat{\mathbf{Z}} = (\hat{z}_1, \ldots, \hat{z}_m)$$

is the parameter vector that minimizes F. Then the covariance matrix $\boldsymbol{V}$ of the estimates $\hat{z}_i$ $(i = 1, \ldots, m)$ is approximately

$$(2.11) \quad \boldsymbol{V} = \boldsymbol{U}^{-1}$$

where $\boldsymbol{U}$ is the matrix of second partial derivatives

$$\boldsymbol{U} = \left(\left(\frac{\partial^2 F}{\partial z_i \partial z_j}(\hat{\mathbf{Z}}) \right) \right)_{i,j=1}^{m}$$

evaluated at the estimate (2.10).

Property 1 is an immediate consequence of (2.6) and (2.7); consequently, as stated earlier, $F(\mathbf{Z})$ and $L(\mathbf{Z})$ are equivalent criteria as far as estimation is concerned. Property 2, proved in Appendix C, shows that $F \geq 0$ and that $F = 0$ only if the predicted and observed catches exactly agree and if the entire initial population is caught. The reader can see by inspection that F, G, and H all vanish when (2.8) and (2.9) are true. But it is not obvious that F is positive otherwise. Since F vanishes only when the initial population is perfectly accounted for by the predicted catches, it is reasonable to consider F a measure of separation between observations and predictions.

It is interesting to compare F with another such measure of separation, namely,

$$(2.12) \quad E(\mathbf{Z}) = \frac{(T_k - \hat{T}_k)^2}{N - T_k} + \sum_{i=1}^{k} \frac{(C_i - \hat{C}_i)^2}{C_i}.$$

This is a chi-square statistic formed by decomposing the initial population N into $k+1$ groups: those caught on the k fishings and those that escape altogether. The sum of k terms in (2.12) is just the usual expression formed by comparing observations and predictions on the k fishings. The remaining term compares the predicted number to escape, $N - T_k$, with the partially observed number $N - \hat{T}_k$. Zippin (1956) discusses applications for E in the context of model 1. Like F, E vanishes when (2.8) and (2.9) are true. But, unlike F, E also vanishes when (2.8) alone is true, regardless of the value of N. All other things being equal, the criterion of minimum F (or maximum likelihood) selects an initial population close to the total catch. Examples later illustrate this "preference" but show that it is not always strong.

Property 3 is based on asymptotic (large N) behavior of the log likelihood, as described in Kendall and Stuart (1979, section 24.7). It can be used to test the null hypothesis that the data conform to a simple model against the alternative hypothesis that they conform to a more complex model with more parameters. For instance, property 3 can be used to reject model 1 in favor of model 2 when F_2' is significantly less than F_1'. Another use of property 2 comes in rejecting the hypothesis that N has a particular value. For instance, if the total catch were 100 fish, one might wish to test the hypothesis that $N = 200$. Values of N that cannot be rejected give an approximate confidence interval for N. Again, this is illustrated in the examples below.

Property 4 is also based on asymptotic behavior of the log likelihood. References include Kendall and Stuart (1979, section 18.26) and Bard (1974, section 7.5). The matrix $\boldsymbol{U}$ of second partial derivatives is called the Hessian. Its inverse $\boldsymbol{V}$ in (2.11) is approximately the covariance matrix of the parameter estimates. Examples below show that the results from this commonly used approximation can be somewhat strange, and even deceptive. More realistic results are obtained by the method described above in connection with property 3.

3. Estimation in Models 1 and 2

Since the estimated parameter vector $\hat{\mathbf{Z}}$ minimizes F, it follows that each component $\hat{z}_i$ of $\hat{\mathbf{Z}}$ satisfies the equation

$$(3.1) \quad \partial F / \partial z_i = 0.$$

Sometimes it is possible to solve (3.1) explicitly for the estimate $\hat{z}_i$. For example, in model 1 the second parameter z_2 is the catchability q_1. The solution to (3.1) turns out to be (as derived by Moran 1951)

$$(3.2) \quad \hat{q}_1(N) = \hat{T}_k \Big/ \left[kN - \sum_{i=1}^{k-1} \hat{T}_i \right].$$

Zippin (1956) and Otis et al. (1978) also take advantage of this result. In (3.2) it is emphasized that $\hat{q}_1$ depends on N; i.e. (3.2) is the best estimate for q_1, given the value of N. If $F(N, q_1)$ represents the function F for model 1, then

$$(3.3) \quad F_1(N) = F[N, \hat{q}_1(N)]$$

is the minimum value of F for model 1 for any given value of N. It follows that the model 1 estimate $\hat{N}$ is the value of N that minimizes F_1 in (3.3). Notice that $F_1(N)$ can be calculated explicitly from the observed catch data $\hat{C}_1, \ldots, \hat{C}_k$ by successive application of formulae (2.2) for $\hat{T}_i$, (3.2) for q_1, (1.8) for C_i, (1.1) for T_i, and (2.7) for F. Thus, the problem of finding $\hat{N}$ for model 1 boils down to the problem of minimizing an explicit function of the variable N. Once $\hat{N}$ is found, then the corresponding estimate $\hat{q}_1(N)$ can be obtained from (3.2).

The minimization problem for $F_1(N)$ has one constraint, namely,

$$(3.4) \quad N \geq \hat{T}_k,$$

since the initial population must be at least as large as the total catch. Let F_1' represent the minimum of F_1 so obtained. Suppose that the true population is N, some number different from $\hat{N}$. By property 3 for F, the statistic $2[F_1(N) - F_1']$ is χ^2 distributed with 1 *df*. With 95% confidence, it must be less than 3.84. This requirement is equivalent to the condition

$$(3.5) \quad F_1(N) \leq F_1' + 1.92.$$

Taken together, the conditions (3.4) and (3.5) define a 95% confidence interval for N. The process of determining this interval, along with the estimate $\hat{N}$, is illustrated in the examples below. Typically, $\hat{N}$ does not lie at the center of the confidence interval.

In case of inadequate data it sometimes happens that (3.5) does not define an upper limit on N. This can happen because $F_1(N)$ has an asymptotic limit as N increases indefinitely:

$$(3.6) \quad F_1(\infty) = \log(\hat{T}_k!) - \hat{T}_k \log(\hat{T}_k) + \hat{T}_k \sum_{i=1}^{k} \hat{C}_i \log(k\hat{C}_i/\hat{T}_k).$$

When the quantity $F_1(\infty)$ in (3.6) is smaller than $F_1' + 1.92$, then arbitrarily large values of N satisfy (3.5), and the data simply do not determine an upper limit on N. This result has important practical consequences illustrated in the examples below.

Explicit results similar to those for model 1 above can also be derived for model 2. In this case

$$\mathbf{Z} = (N, q_1, q)$$

and, based on (3.1), it turns out that

$$(3.7) \quad \hat{q}_1(N) = \hat{C}_1/N$$

$$(3.8) \quad \hat{q}(N) = (\hat{T}_k - \hat{C}_1) / [(k-1)(N - \hat{C}_1) - \sum_{i=1}^{k-1} (\hat{T}_i - \hat{C}_1)].$$

In (3.7)–(3.8), as in (3.2), it is emphasized that the catchability estimates depend on a given value of N. It follows that the function

$$(3.9) \quad F_2(N) = F[N, \hat{q}_1(N), \hat{q}(N)]$$

represents the minimum value of F for model 2 for any given value of N. The sequence of formulae (2.2) for $\hat{T}_i$, (3.7) for q_1, (3.8) for q, (1.12) for C_i, (1.1) for T_i, and (2.7) for F allows $F_2(N)$ to be computed explicitly from the observed catch data.

The use of F_2 for model 2 exactly parallels the use of F_1 for model 1. $F_2(N)$ takes its minimum value F_2' at the model 2 estimate for N. Also, the conditions (3.4) and

$$(3.10) \quad F_2(N) \leq F_2' + 1.92$$

define a 95% confidence interval for N in model 2. The condition (3.10), like (3.5), may fail to define an upper limit for N. This happens when the asymptotic limit of F_2

$$(3.11) \quad F_2(\infty) = \log(\hat{T}_k!) - \hat{T}_k \log(\hat{T}_k) + \hat{T}_k + \sum_{i=2}^{k} \hat{C}_i \log\left(\frac{(k-1)\hat{C}_i}{\hat{T}_k - \hat{C}_1}\right)$$

is smaller than the right-hand side of (3.10).

By property 3 for F, the statistic $2(F_1' - F_2')$ is χ^2 distributed with 1 *df* when model 1 is true. Consequently, this quantity exceeds 3.84 only 5% of the time by chance, and model 1 can be rejected with 95% confidence in favor of model 2 when

$$(3.12) \quad F_1' \leq F_2' + 1.92.$$

Thus, (3.12) is a condition for deciding that the first fishing has a significantly different catchability from the others.

All analytical results described in this section are proved in Appendix D. The statistical tests outlined above are based on asymptotic properties of the log likelihood and are therefore approximate. Thus, they should be regarded somewhat informally. The examples below illustrate how the methods give results consistent with an intuitive look at the data. Practitioners are encouraged always to go back to the raw catch data when interpreting an analysis based on the methods here.

4. Features of Likelihood Estimates

The likelihood L for removal data is derived from probabilities associated with finite samples, and these probabilities depend on the sample size. Consider a simple illustration. If a perfectly balanced coin is tossed an even number of times, the best (most likely) prediction before any tosses are made is the the number of heads will equal the number of tails. In the case of 2 tosses, there is a 50% chance that this prediction will come true. With 10 tosses, the probability drops to 24.6%; with 20 tosses, it drops further to 17.6%; with 50 tosses, it is only 11.2%. Clearly, the chances are rather small of getting exactly a 25/25 split between heads and tails in 50 tosses, although any other split is even less likely. Since the removal method is related to a coin tossing experiment in which the number N of tosses is unknown, the maximum likelihood estimate will favor a small N, as described above in connection with property 2 for F.

This feature of likelihood estimation conflicts somewhat with a strictly deterministic view in which sample size has nothing to do with the problem. From that point of view, only the proportionality of catches matters. For instance, the example in the introduction involves three catches: 90, 60, and 40. As described earlier, the trend in catch suggests an initial population of 270 with a capture probability of 1/3 (0.333). However, the model 1 estimate for the data just given is

$$(4.1) \quad (\hat{N}, \hat{q}_1) = (265.2, 0.342)$$

where $F = 2.925$. When N is 270 and q_1 is 1/3, the value of F is 2.935, slightly larger than the minimum. Thus, the maximum likelihood estimate for N includes about five fewer fish than the estimate that perfectly explains the data on the basis of a deterministic model. In general, likelihood estimates

TABLE 1. Catches of brook trout in the Nashwaak Experimental Watersheds Project, 1974–76, for the 16 samples with total catch exceeding 40. These come from Upper Narrows Mountain Brook (UNM) and Hayden Brook (HAY). The final sample 17 corresponds to the first four trappings in a sample investigated by Leslie and Davis (1939) and Moran (1951).

Sample	Stream	Section	Date	Catch at fishing No. 1	2	3	4	Total catch
1	UNM	2	July 1974	45	11	18	8	82
2	UNM	3	July 1974	52	31	10	12	105
3	UNM	4	July 1974	48	10	6	6	70
4	UNM	2	July 1976	88	22	11	2	123
5	UNM	3	July 1976	50	10	8	6	74
6	UNM	4	July 1976	44	12	1	0	57
7	UNM	2	Oct. 1975	53	22	14	4	93
8	HAY	4	July 1974	24	13	5	3	45
9	HAY	1	July 1976	37	4	2	3	46
10	HAY	2	July 1976	63	9	12	4	88
11	HAY	3	July 1976	16	22	6	4	48
12	HAY	4	July 1976	47	16	11	4	78
13	HAY	6	July 1976	38	11	4	5	58
14	HAY	2	Oct. 1974	18	16	5	2	41
15	HAY	4	Oct. 1974	27	9	5	3	44
16	HAY	3	Oct. 1974	55	6	3	4	68
17	—	—	—	49	32	31	34	146

have Feature 1: *if the observed catch data can be explained perfectly by a deterministic model, i.e. if (2.8) holds exactly, then the maximum likelihood parameter estimates may not correspond to this explanation unless (2.9) is also true.*

The significance of the likelihood "preference" for small N can also be seen by considering the effect of reducing the catch data by a fixed factor. For example, consider the problem of analyzing the three catches: 9, 6, 4. These follow a progression identical to that in the example above, since each catch has been divided by 10. An initial population of 27 with a capture probability of 1/3 perfectly explains these data. From the comments above, it is not surprising that the likelihood estimate $\hat{N}$ is not 27, but is it 1/10 of 265.2? The answer is no; the parameter estimates are

$$(4.2) \qquad (\hat{N},\hat{q}_1) = (23.2,\ 0.417)$$

where $F = 1.682$. When N is 27 and q_1 is 1/3 (the perfect deterministic scenario) the value of F is 1.795. When N is 26.52 and q_1 is 0.342, as one might conjecture from (4.1), F is 1.774. This illustrates Feature 2: *if the catch data are scaled by a factor, then the likelihood estimate for* N *may not be scaled accordingly.*

Feature 2 may have practical implications. What if fish swim in schools of 10 and are only caught in schools? Then the catches of 90, 60, and 40 really represent catches of 9, 6, and 4 units, and from (4.2) the likeliest initial population is 23.2 units, or 232 fish. This result differs notably from the estimated 265 fish in (4.1) obtained by assuming that each fish is a unit by itself.

Finally, consider the sample of four catches: 1000, 90, 60, and 40. These data cannot be obtained from a deterministic model 1 because the first catch is too large. (An initial catch of 135 would fit the remaining progression perfectly.) However, the data do exactly fit a deterministic model 2 with

$$(N,q_1,q) = (1270,\ 0.787,\ 0.333)$$

where q_1 is just the ratio 1000/1270. In this scenario, the first capture probability precisely explains the first catch; the remaining population of 270 fish is just right to give a deterministic model 1 fit for the last three catches. Not surprisingly, given the discussion above, this is not the model 2 likelihood estimate; but one might suppose that the estimate for N is 1265.2, i.e. the model 1 estimate (265.2) for the last three catches plus the first catch (1000). However, for the sequence of four catches above, the model 2 estimate turns out to be

$$(\hat{N},\hat{q}_1,\hat{q}) = (1263.8,\ 0.791,\ 0.345).$$

In particular, the estimate for N is slightly less than 1265.2. In this example, the difference is small because the final 3 catches follow an exact progression. Noisier data would give a more marked difference. The general principle here is Feature 3: *the model 2 estimate for* N *from* k *observed catches may not be the first catch plus the model 1 estimate for the final* k−1 *catches.*

Feature 3 shows that model 2 is not simply a trivial modification of model 1. This is perhaps a little surprising, since it would seem that the experimenter could regard the population after the first catch as a fresh starting point and simply apply model 1 thereafter. Indeed, the capture probability estimates (3.7)–(3.8) suggest this, particularly when one compares (3.8) with (3.2). However, even the first catch results from a statistical process, and for optimal use of the data, it should be considered jointly with the others.

5. Examples

I illustrate the above theory with data from work performed in connection with the Nashwaak Experimental Watersheds

TABLE 2. Parameter estimates and minimum F values (models 1 and 2) for the samples in Table 1, as well as the full sample (LDM) investigated by Leslie and Davis (1939) and Moran (1951). The χ^2 statistic, equal to twice the difference of the F values, indicates that model 1 should be rejected in favor of model 2 when χ^2 exceeds 3.84. In such cases, a plus or a minus shows whether the capture probability increases or decreases, respectively. The samples are categorized into types A, B, C, or D, depending on the nature of the model selected.

	Model 1			Model 2						
Sample	$\hat{N}$	$\hat{q}_1$	F	$\hat{N}$	$\hat{q}_1$	$\hat{q}$	F	χ^2	+/−	Type
1	90.6	0.44	6.95	123.6	0.36	0.19	4.58	4.74	−	C
2	114.6	0.46	3.92	114.2	0.46	0.46	3.91	0.02		A
3	71.1	0.61	4.71	78.3	0.61	0.34	2.22	4.98	−	C
4	123.4	0.70	1.81	123.8	0.71	0.67	1.71	0.20		A
5	75.5	0.60	4.93	84.6	0.59	0.32	2.18	5.50	−	C
6	57.0	0.80	1.12	57.0	0.77	0.93	0.08	2.08		A
7	96.4	0.55	2.18	96.3	0.55	0.56	2.18	0.00		A
8	46.6	0.54	1.43	45.7	0.52	0.61	1.22	0.42		A
9	46.0	0.73	4.45	47.9	0.77	0.39	1.92	5.06	−	C
10	88.9	0.64	6.99	97.0	0.65	0.35	3.49	7.00	−	C
11	55.0	0.39	5.49	48.7	0.33	0.66	1.52	7.94	+	B
12	80.3	0.57	2.14	81.6	0.58	0.51	1.97	0.34		A
13	58.9	0.61	3.06	61.2	0.62	0.46	2.32	1.48		A
14	43.2	0.50	2.90	41.0	0.44	0.72	0.69	4.42	+	B
15	44.8	0.58	1.52	45.5	0.59	0.52	1.40	0.24		A
16	68.0	0.74	5.87	71.6	0.77	0.37	2.12	7.50	−	C
17	344.1	0.13	4.44	∞	—	—	3.48	1.92		D
LDM	519.0	0.08	12.18	544.6	0.09	0.07	10.65	3.06		A

Project, 1974–76, in New Brunswick, Canada. As part of this project, Upper Narrows Mountain Brook (in four sections), Lower Narrows Mountain Brook (in four sections), and Hayden Brook (in six sections) were sampled by electrofishing. Nets to confine the population were placed above and below a section while it was fished, and each section was sampled from one to three times per year. Typically, a sample consisted of four electrofishings; stunned fish were removed from the population after each fishing and replaced only after all fishings were completed. Three to four species of fish were usually caught in this way. Here, I present the data for brook trout (*S. fontinalis*) from every sample in which at least 40 fish were caught. From a total of 99 available trout samples, this selection criterion gives numbers 1 to 16 listed in Table 1. Notice, incidentally, that none of the selected samples comes from Lower Narrows Mountain Brook; all samples from this stream included a catch of less than 40 fish each.

The final sample 17 in Table 1 comprises the first four trappings of rat (*Rattus rattus*) in an example discussed by Leslie and Davis (1939) and Moran (1951). The full sample includes 18 successive trappings with catches of 49, 32, 31, 34, 16, 33, 22, 27, 17, 19, 18, 16, 18, 12, 14, 12, 17, and 7, giving a total catch of 394 animals. These were taken from a 22.5-acre (1 acre = 0.4 ha) area in Freetown, Sierra Leone. This is the sample about which Moran is quoted in the introduction; he finds slight evidence of trap-shyness after the first trapping, due to the rather large first catch relative to the subsequent trend.

Analysis of these data requires a number of computational tools. The earlier discussion of theory implicitly assumes that problems such as function minimization and Hessian calculation can be solved. Appendix E gives explicit details on the numerical solution of these problems. Although the calculations would be rather hopeless by hand, they can readily be accomplished on a microcomputer. For example, using general purpose software by Schnute (1982), all that is additionally required here is a program to calculate $F(\mathbf{Z})$.

Table 2 lists the model 1 and 2 estimates and minimum F values, F_1' and F_2', for the 17 samples in Table 1, as well as the full sample of 18 trappings discussed in the previous paragraph. The statistic $2(F_1' - F_2')$ is also shown. Based on property 2 for F, model 1 can be rejected in favor of model 2 when this statistic exceeds 3.84; see also (3.12). This occurs in 8 of the 16 trout samples, far more frequently than the 5% chance occurrence one would expect if model 1 applied to all samples. In 6 of the 8 samples where model 2 is indicated, the catchability decreases after the first fishing; in the other 2 samples it increases. This latter phenomenon, the apparent opposite of trap-shyness, might be explained if the first fishing rouses fish from inaccessible locations and thus makes them more available for capture later.

Stated somewhat more precisely than in Table 2, the model 1 estimate for the complete rat sample is

$$(\hat{N}, \hat{q}_1) = (518.98,\ 0.0759),$$

a result to be compared with the estimate (520, 0.0756) reported by Moran (1951). The slight difference is due to the fact that Moran used an approximate method and, even then, carried his approximation only through a few iterations. In any case, the value 3.06 of the χ^2 statistic, since it is less than the critical 3.84, justifies Moran's use of model 1; however, the value is large enough also to make one slightly suspicious, as Moran was. Notice in Table 2 that the population estimate based only on the first 4 trappings (sample 17) is about 344,

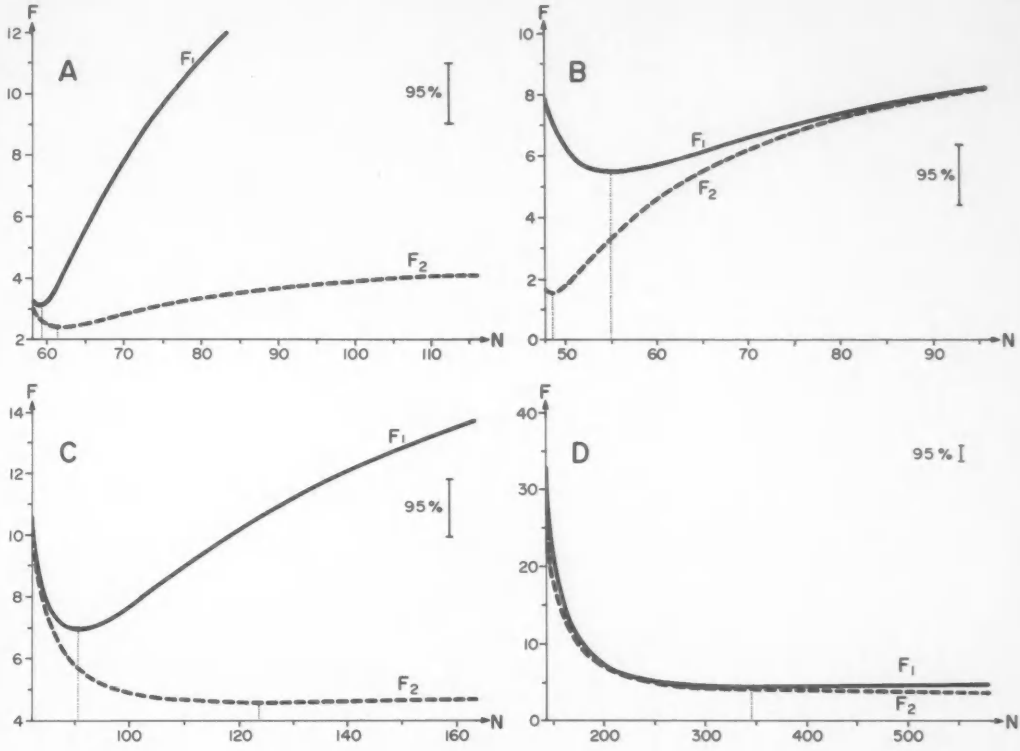

FIG. 1. Separation statistic $F(N)$ for model 1 (solid curve, F_1) and model 2 (broken curve, F_2). At each N, F is evaluated at optimum values of capture probability for the given model. The estimate $\hat{N}$ corresponds to the minimum value of F, as indicated by a vertical dotted line to each curve. The 95% vertical confidence interval represents the difference between minimum F values for models 1 and 2, respectively, to justify rejecting model 1 in favor of model 2. Figures 1A, 1B, 1C, and 1D apply to samples 13, 11, 1, and 17, respectively, in Table 1. In each figure, N ranges from the total observed catch $\hat{T}_L$ on the left to twice (Fig. 1A, 1B, 1C) or four times (Fig. 1D) that amount on the right.

notably less than 519. As shown below, however, the estimate of 344 is very poorly determined. Model 2 gives the rather startling estimate of ∞ in this case. Essentially, this results from the fact that the second, third, and fourth catches (32, 31, 34) suggest, if anything, an increasing trend.

Model 3 is not beneficial for analysis of any of the samples examined here. Typically, the optimum model 3 scenario, subject to the condition $0 \leq a \leq 1$, turns out to be model 2, i.e. the case $a = 0$. In cases where this is not true, then model 3 does not give a significant improvement. For example, the estimates for sample 13 turn out to be

$$(\hat{N},\hat{q}_1,\hat{q},\hat{a}) = (61.7,\ 0.616,\ 0.433,\ 0.107)$$

with a minimum F value of 2.31, compared with 2.32 for model 2.

The best way to understand what conclusions can be drawn from models 1 and 2 for removal data is to plot the functions $F_1(N)$ and $F_2(N)$ defined in (3.3) and (3.9). When this is done for all the samples presented here, four characteristic types of data sets can be identified. The first, shown in Fig. 1A, is illustrated by sample 13. The F_2 curve for model 2 lies below the F_1 curve for model 1, as it should due to the extra parameter in model 2. However, the minimum of F_2 is not far enough (a distance indicated by the 95% vertical confidence interval) below the minimum of F_1 to reject model 1. This is fortunate from the experimenter's point of view because the F_1 minimum is the more sharply defined; the F_2 curve is flatter and rises less steeply from its minimum. We refer to a sample with the characteristics just described as "type A."

Sample 11 (Fig. 1B) illustrates a "type B" data set. Here, the F_2 minimum is far enough below that of F_1 to justify rejecting model 1. Again, this works to the experimenter's advantage because the rejected model has a less sharply defined minimum. This is also true for sample 14, and in both cases model 2 indicates an increased capture probability after the first fishing (see Table 2). As a result, the model 2 estimate for N is smaller than that for model 1.

Figure 1C, obtained from sample 1, corresponds to a "type C" data set. Again, model 1 can be rejected; however,

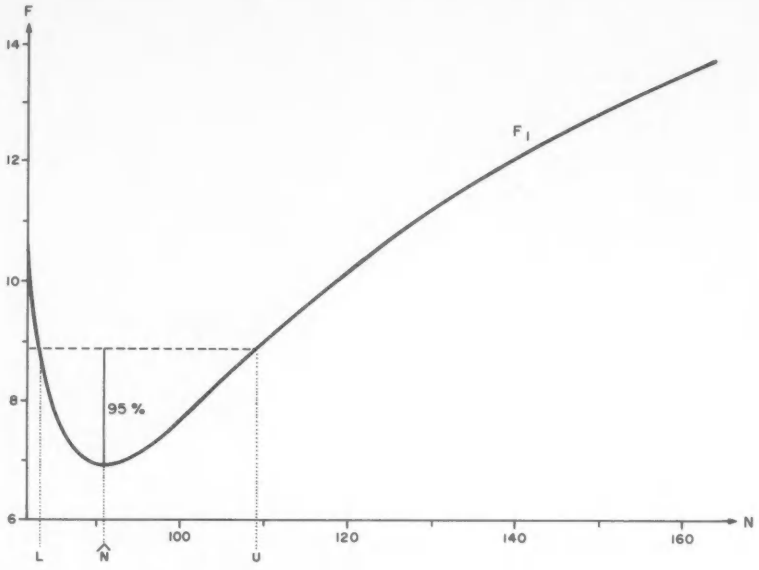

FIG. 2. $F_1(N)$, as in Fig. 1C for model 1. Again, $\hat{N}$ is determined by the minimum value of F_1. Also, lower (L) and upper (U) confidence limits on N are located by restricting F_1 to values less than the 95% confidence interval above the minimum F_1.

this rejection leaves the experimenter much worse off than would be supposed on the basis of model 1. Notice that the F_2 curve in Fig. 1C is so flat that the minimum can hardly be identified visually. On the other hand, the F_1 curve has a nicely defined minimum. The model 2 estimate for N is larger than that for model 1, as is the case in all type C samples in Table 2. This larger estimate is consistent with the decreased capture probability after the first fishing, as indicated by model 2. Just as Moran anticipated, increasing trap-shyness causes the model 1 estimate to be too low.

Finally, Fig. 1D, based on sample 17, illustrates "type D" characteristics. Here, model 1 cannot be rejected, but the minimum for F_1 is very poorly defined. Consequently, type D is similar to type C, except that in this case even model 1 indicates that the experimenter is in a poor position to estimate N. Notice that in the particular case of sample 17, the function F_2 continues to decrease throughout the range of N shown in Fig. 1D. In fact, this decrease continues for arbitrarily large N and gives the estimate $\hat{N} = \infty$, as mentioned earlier.

The above paragraphs refer frequently to estimates for N, which are well or poorly defined. Figure 2 illustrates the detailed statistical analysis behind such references. Property 3 for F, along with conditions (3.4), (3.5), and (3.10), gives the theoretical basis for the construction in Fig. 2. The 95% vertical confidence interval (1.92 units of change in F) specifies the amount of change in F above the minimum required to reject a value of N. In this case, the F_1 curve for sample 1 is shown. Since the minimum F_1' is 6.95 here, values of N can be excluded for which

$$F_1 > 6.95 + 1.92 = 8.87.$$

This leaves values of N between 83.4 and 109.2, the confidence interval for sample 1, model 1, shown in Table 3. Similarly, Table 3 shows confidence intervals for all samples, models 1 and 2, obtained in this way. (Appendix E describes the numerical counterpart to the analysis illustrated graphically here.) Sometimes, in cases indicated with an asterisk, condition (3.4) defines the lower limit; i.e. the value of F at the total catch is still less than the cutoff level, so the total catch ultimately limits the population estimate. In all type C samples, the model 2 upper limit obtained from (3.10) is ∞. This happens, as inspection of Fig. 1C suggests, because the limiting value of F_2 in (3.11) is less than the cutoff level. Similarly, in type D the F_1 curve is confined below the cutoff.

Table 3 also shows the standard deviation of $\hat{N}$ for models 1 and 2 obtained by the inverse Hessian method described in property 4. The method actually gives a complete covariance matrix, and there is some risk in looking at just one of the standard deviations because of correlations among the parameter estimates. Bard (1974, p. 172–173) gives an illuminating example. It is desirable to choose parameters whose estimates are as uncorrelated as possible. In model 1, for example, the parameters N and q_1 have negatively correlated estimates, because a larger N requires a smaller q_1 to explain the same catches. However, the parameters N and C_1 turn out to have less correlated estimates. For instance, here are the correlations for sample 1:

TABLE 3. Confidence intervals for the initial population N in models 1 and 2. These are determined, as in Fig. 2, from critical values for F. Lower limits marked with an asterisk correspond to the observed total catch, with an F value below the critical level. Standard deviations (SD) for $\hat{N}$ determined from the inverse Hessian of F with respect to model parameters are also shown. In cases marked —, the estimate $\hat{N}$ lies on the boundary of admissible values, and the inverse Hessian method is inapplicable.

	Model 1			Model 2		
Sample	$\hat{N}$	Conf. int.	SD	$\hat{N}$	Conf. int.	SD
1	90.6	83.4−109.2	5.6	123.6	87.3−∞	54.6
2	114.6	107.0−131.5	5.6	114.2	106.0−147.9	7.2
3	71.1	70.0*−76.3	1.6	78.3	70.0*−∞	10.3
4	123.4	123.0*−126.7	1.1	123.8	123.0*−130.5	1.6
5	75.5	74.0*−81.3	1.8	84.6	74.3−∞	12.8
6	57.0	57.0*−58.0	—	57.0	57.0*−57.5	—
7	96.4	93.0*−104.8	2.8	96.3	93.0*−111.6	3.5
8	46.6	45.0*−54.4	2.1	45.7	45.0*−56.5	1.8
9	46.0	46.0*−47.7	—	47.9	46.0*−∞	4.2
10	88.9	88.0*−93.4	1.5	97.0	88.1−∞	10.4
11	55.0	48.4−81.6	5.8	48.7	48.0*−55.7	1.6
12	80.3	78.0*−87.3	2.3	81.6	78.0*−105.4	4.0
13	58.9	58.0*−64.0	3.1	61.2	58.0*−130.9	4.4
14	43.2	41.0*−53.5	2.6	41.0	41.0*−45.5	—
15	44.8	44.0*−50.5	1.5	45.5	44.0*−78.4	2.8
16	68.0	68.0*−69.9	—	71.6	68.0*−∞	5.9
17	344.1	211.0−∞	141.5	∞	232.8−∞	∞
LDM	519.0	468.2−606.7	40.0	544.6	497.3−673.9	44.5

$$\text{corr}\,(\hat{N},\hat{q}_1) = -0.82$$

$$\text{corr}\,(\hat{N},\hat{C}_1) = -0.57.$$

To avoid correlation problems as much as possible, the parameters N and C_1 in model 1 and N, C_1, and C_2 in model 2 are used in the covariance analysis leading to the standard deviations shown in Table 3.

Incidentally, the method also gives estimated standard deviations for parameters other than N. In the example just cited (model 1, sample 1), the second parameter can be either q_1 or C_1. The corresponding estimates and standard deviations are shown below:

Parameter	Estimate	Standard deviation
q_1	0.437	0.064
C_1	39.6	4.0

Such results are usually much less important than the estimated standard deviation of $\hat{N}$ and, for brevity, are not generally tabulated here.

One difficulty is immediately apparent with the Hessian method: it sometimes fails because the estimate $\hat{N}$ lies on the boundary of admissible values. For instance, in sample 6, both models 1 and 2 give the estimate $N = 57$, the total catch. A plot of F_1 and F_2 shows that this value of N does not correspond to a local minimum of F in either case but simply to the lowest value of F, subject to the condition that N be at least 57. The theory behind property 4 assumes that the derivative of F must vanish at the estimate, as in (3.1). Earlier work on the removal method fails to address this problem.

The inverse Hessian method suffers from a still deeper problem. Consider sample 16. In this case, model 1 is rejected, and based on the standard deviation of $\hat{N}$, Table 3 shows that

$$N = 71.6 \pm 11.6$$

with 95% confidence. (Here, 11.6 is the product of the standard deviation 5.9 times 1.96, the 95% two-tailed limit for the normal distribution.) The implied lower limit of 60.0 is certainly too low because the total catch for sample 16 is 68. The upper limit of 83.2 is even more deceptive. For this type C sample, the F_2 curve is extremely flat: the minimum is 2.12 (Table 2) and the asymptotic limit (from equation (3.11)) is 3.55. From the point of view of the likelihood function, the ultimate criterion used to determine the estimate, any value of N above 68 looks acceptable, i.e. gives an F value below the cutoff of 4.04 (2.12 + 1.92). In short, the inverse Hessian method appears to give a lower confidence limit too far from the estimate 71.6 and an upper limit too close to it.

The curves in Fig. 1 lend insight into the reason why the inverse Hessian method fails for the removal problem. The method essentially is based on a quadratic approximation to the negative log likelihood. If the function F really were quadratic, then (i) it would be symmetric around the minimum, (ii) it would always have upward curvature, and (iii) it would become arbitrarily large as the parameters move away from their estimated values. As Fig. 1 shows, all these conditions are violated. First, F is steeper and more sharply curved to the left of $\hat{N}$ than to the right. Second, the right-hand

TABLE 4. Three scenarios for the population underlying sample 16. The first and second scenarios correspond to the optimal estimates for models 1 and 2, respectively. The third scenario is optimal for model 2, given that the initial population is 1 million.

Model	F value	Fish. No.	Population	q	Pred. C	Obs. C
1	5.87	1	68.00	0.74	50.26	55
		2	17.74	0.74	13.11	6
		3	4.63	0.74	3.42	3
		4	1.21	0.74	.89	4
2	2.12	1	71.59	0.77	55.00	55
		2	16.59	0.37	6.20	6
		3	10.39	0.37	3.88	3
		4	6.51	0.37	2.43	4
2	3.56	1	1 000 000.00	0.0000550	55.00	55
		2	999 945.00	0.0000043	4.33	6
		3	999 940.67	0.0000043	4.33	3
		4	999 936.33	0.0000043	4.33	4

portion of the curve has an inflection point and ultimately has downward curvature. Finally, F has a finite limit for large values of N. The fact that F is steeper than expected on the left and flatter than expected on the right accounts for the peculiar results from the inverse Hessian method described above. These problems could be partially remedied by using log (N), rather than N, as the parameter, but this approach is not explored here because the method based on property 3 is preferable in any case.

It is not merely an academic point that sample 16 does not ultimately lead to a reasonable confidence interval for N. There is a perfectly common-sense reason why this is so. The sample comprises the four catches: 55, 6, 3, and 4 (Table 1). Quite obviously, the first catch is very large relative to the subsequent trend. Ignoring it, we see the trend 6, 3, and 4. How much longer could an experimenter continue catching 3 or 4 fish? Surely it is not clear on the basis of these data; perhaps hundreds of times. If model 1 is to be believed, the 95% confidence interval for N is between 68 and 69.9 fish; i.e. only about 2 fish remain to be caught. Is that believable from the data? Table 4 shows the optimal scenario for predicted catches based on models 1 and 2. Clearly, model 2 gives a more sensible description, but so does model 2 starting with 1 million fish. In that scenario, the huge initial population could continue to be fished for a long time, taking about 4 fish on each fishing. An experimenter can reasonably reject such a possibility only on the basis of information beyond the actual catch data. Thus, the seemingly peculiar statistical result points to a genuine experimental problem in sample 16, and similarly in all type C and D samples presented here.

This discussion shows that a rigid belief in model 1 can be nothing more than an exercise in self-deception, particularly if one believes the typically tight confidence limits derived from that model. The experimenter should be particularly careful of the (rather common) practice of using just two fishings. Although this is theoretically enough to give model 1 estimates, often with tight confidence, it certainly is not enough to address the more crucial issue of model validity. As the examples here illustrate, it is sometimes necessary to continue fishing to get reasonable confidence in model estimates.

Usually, the experimenter cannot plot the functions F_1 and F_2 in the field to decide whether or not there is enough data. One way to inspect the data intuitively is to focus on the escapement fraction implied by the catch. In sample 16, for instance, the first two catches (55, 6), if they are proportional to current populations, imply that only

$$p_1 = 6/55 = 10.9\%$$

escaped the first fishing. The second two catches (6,3) raise this estimate to $p_2 = 50\%$, and the third pair (3,4) to an impossible $p_3 = 133\%$. Table 5 shows such estimates for all samples, along with the geometric mean p between p_2 and p_3 as a rough estimate of escapement fraction based on the last three fishings. All type C samples, like sample 16, show a large jump in this fraction, as indicated by the ratio p/p_1. In fact, for these data, the sample is type C if and only if $p/p_1 > 3$. After only three fishings, the ratio p_2/p_3 also proves to be a reasonably good indicator when it exceeds 3. Excluding sample 10, Table 5 shows that the sample is type C or D if and only if $p > 0.75$. In general, if the catch trend indicates that over 3/4 of the fish continue to escape on each fishing, or if it appears that the escapement fraction jumps by a factor of more than 3 after the first fishing, the experimenter seems well advised to continue beyond four fishings.

6. Alternative Models

Since numerous methods have been proposed for analyzing removal data, it is appropriate to compare the methods here with those described elsewhere. Leslie and Davis (1939) utilized a model based on equation (1.2). They implicitly assume that the observed catch $\hat{C}_i$ is determined by

$$(6.1) \quad \hat{C}_i = q_i(N - \hat{T}_{i-1}) + \sigma\epsilon_i$$

where the ϵ_i, $i = 1,\ldots,k$, are independent normal random variables with mean 0 and standard deviation 1, and σ is the standard deviation of $\hat{C}_i$. In the case of model 1, where each q_i is equal to q_1, (6.1) implies that the parameters q_1N and $-q_1$ can be estimated as the intercept and slope, respectively, of the line determined by regressing $\hat{C}_i$ on $\hat{T}_{i-1}$. Unfortunately,

TABLE 5. Rough estimates of the escape probability associated with the first fishing (p_1), second fishing (p_2), third fishing (p_3), and last three fishings (p). After three fishings, large values of p_2 or the ratio p_2/p_1 suggest that it may be impossible to find a statistical upper limit for the initial population. A similar problem is indicated after four fishings if p or p/p_1 is large.

Sample	p_1	p_2	p_3	p	p_2/p_1	p/p_1	Type
1	0.24	1.64	0.44	0.85	6.69	3.49	C
2	0.60	0.32	1.20	0.62	0.54	1.04	A
3	0.21	0.60	1.00	0.77	2.88	3.72	C
4	0.25	0.50	0.18	0.30	2.00	1.21	A
5	0.20	0.80	0.75	0.77	4.00	3.87	C
6	0.27	0.08	0.00	0.00	0.31	0.00	A
7	0.42	0.64	0.29	0.43	1.53	1.03	A
8	0.54	0.38	0.60	0.48	0.71	0.89	A
9	0.11	0.50	1.50	0.87	4.63	8.01	C
10	0.14	1.33	0.33	0.67	9.33	4.67	C
11	1.38	0.27	0.67	0.43	0.20	0.31	B
12	0.34	0.69	0.36	0.50	2.02	1.47	A
13	0.29	0.36	1.25	0.67	1.26	2.33	A
14	0.89	0.31	0.40	0.35	0.35	0.40	B
15	0.33	0.56	0.60	0.58	1.67	1.73	A
16	0.11	0.50	1.33	0.82	4.58	7.48	C
17	0.65	0.97	1.10	1.03	1.48	1.58	D

it sometimes happens that this procedure gives an estimate for N less that the total catch $\hat{T}_k$. This is not too surprising, since (6.1), unlike the binomial model of this paper, allows $\hat{C}_i$ to be less than 0 or greater than the available population $N - \hat{T}_{i-1}$. On the other hand, (6.1) does have one attractive feature in common with the binomial model: it satisfies (2.5); i.e. $\hat{C}_i$ has a reasonable expected value.

Cross and Scott (1975) presented an analysis for model 2 along the lines of Leslie and Davis (1939). This method can also be motivated by (6.1). Since $q_i = q$ for $i > 2$, the regression of $\hat{C}_i$ on $\hat{T}_{i-1}$ simply omits the first catch (with $i = 1$). Strangely, Cross and Scott then failed to get the rest of the estimation correct. Their graphical method implicitly assumed that

$$\hat{C}_1 = \hat{q}\hat{N} \tag{6.2}$$

rather than

$$\hat{C}_1 = \hat{q}_1\hat{N} \tag{6.3}$$

as would be consistent with (6.1) and model 2. For example, given the four catches 1000, 90, 60, and 40, Cross and Scott would use the final three to obtain $\hat{q} = 1/3$ and then apply (6.2) to derive the estimate $\hat{N} = 3000$. A correct application of (6.1) would give estimates $\hat{q} = 1/3$ and $\hat{N} = 1270$ by regression, and then $\hat{q}_1 = 0.787$ (i.e. 1000/1270) from (6.3).

DeLury (1947) gave a method based on (1.4), assuming log-normal errors. Taking logs and including an error term, (1.4) becomes

$$\log(\hat{C}_i) = \log(q_i N) + \sum_{j=1}^{i-1} [\log(1 - q_j)] + \sigma\epsilon_i. \tag{6.4}$$

When model 1 applies, (6.4) reduces to

$$\log(\hat{C}_i) = \log(q_1 N) + [\log(1 - q_1)](i - 1) + \sigma\epsilon_i, \tag{6.5}$$

which suggests an estimation method for model 1 based on regressing $\log(\hat{C}_i)$ on $i - 1$, the effort (i.e. number of fishings) prior to the ith catch. Like (6.1), the model (6.4) also leads to regression estimates for the parameters of model 2.

The DeLury model (6.4) is in one sense better than the Leslie–Davis model (6.1): it does not allow negative catches C_i, since the errors are log-normal. However, a significant weakness of the DeLury model is that it predicts the catch $\hat{C}_i$ without reference to the previous total catch $\hat{T}_{i-1}$. Instead, it uses only the predicted catch C_i prior to any fishing. By contrast with the binomial and Leslie–Davis models, which share the property (2.5), the DeLury model (6.4) implies that

$$E\{\hat{C}_i\} = C_i \exp(\sigma^2/2) \tag{6.6}$$

where C_i is determined by the expression (1.4). The factor $\exp(\sigma^2/2)$ appears in (6.6) as a result of properties of the log-normal distribution (Aitchison and Brown 1957, p. 9). Incidentally, C_i is the median of the distribution of $\hat{C}_i$.

An interesting potential compromise between the Leslie–Davis model (6.1) and the DeLury model (6.4) would be a variant of (6.1) with log-normal errors:

$$\log(\hat{C}_i) = \log[q_i(N - \hat{T}_{i-1})] + \sigma\epsilon_i. \tag{6.7}$$

Even better, one might assume that the distribution of error ϵ_i in (6.1) is such that $\hat{C}_i$ can only take values between 0 and $N - \hat{T}_{i-1}$. Such statistical assumptions sacrifice the technical convenience of estimation by linear regression for models 1 and 2, but they are certainly closer to reality. To my knowledge, no one has ever studied these possibilities.

The most comprehensive approach to models based on the

binomial distribution appears in the monograph by Otis et al. (1978). They contemplated models 1 and 2 of this paper, and even (p. 44) a version of model 3. They also discussed a general model in which the q_i values are random, following some parametric distribution. Although they considered an approach to confidence intervals similar to that based on property 3 (their Appendix O), they rejected this approach in favor of one based on the inverse Hessian (2.11) in property 4. They did not develop the properties of the likelihood function as fully as is done here or pursue a discussion of models 1 and 2 along the lines of Table 2 and Fig. 1 above.

This paper emphasizes primarily the importance of model 2 in analysis of removal data, since practitioners traditionally have been most concerned that the first fishing might be exceptional. Future work might well take account of alternative statistical models, such as (6.7); also, the model of Otis et al. (1978) in which the q_i values are random may prove to be important in some cases. No matter what model is used, considerations similar to those by which data sets of types A, B, C, and D are identified here will surely be important in deciding whether or not the first fishing needs to be singled out from the rest. If so, as the examples here illustrate, further data may be needed to determine the population with reasonable confidence.

Acknowledgments

I am very grateful to Dr P. E. K. Symons for his active participation throughout this project. He supplied the stream data analyzed here and expressed deep concern that classical methods seemed inappropriate. He introduced me to much of the literature on this problem and suggested something along the lines of model 2 as an alternative. Without his ongoing encouragement, I would never have undertaken this work, let alone completed it. I am also grateful to Dr D. Fournier and Dr B. A. Megrey for valuable critical comment on both the organization and content of an earlier draft. In particular, Dr Fournier suggested (6.7) as an interesting prospect for future investigation.

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Appendix A. Notation

This appendix lists the paper's notation in alphabetical order, with Greek symbols last. In general, a circumflex over a letter indicates an observation or parameter estimate. A boldface-type letter is either a vector or a matrix.

a	parameter in model 3; describes rate at which q_i tends to q
$\hat{a}$	estimate for a
$C_i(\mathbf{Z})$	predicted catch on fishing i ($i = 1,\ldots,k$) prior to any fishings
$\hat{C}_i$	observed catch on fishing i ($i = 1,\ldots,k$)
$E(\mathbf{Z})$	a chi-square statistic measuring separation between C_i and $\hat{C}_i$
$\mathrm{E}\{\cdots\}$	expected value of a statistic or random variable
$F(\mathbf{Z})$	$-\log L(\mathbf{Z})$, except for a constant; another separation statistic. Incidentally, values of the function F need not conform to the statistical F distribution.
$F_1(N)$	F in model 1 at optimum catchability for given N
F_1'	minimum value of $F(N,q_1)$ in model 1
$F_2(N)$	F in model 2 at optimum catchabilities for given N
F_2'	minimum value of $F(N,q_1,q)$ in model 2
$G(\mathbf{Z})$	a component of F associated with the uncaught population
$H(\mathbf{Z})$	a component of F associated with predicted and observed catches
k	number of fishings or trappings in a removal experiment
$L(\mathbf{Z})$	likelihood for a removal sample
m	number of model parameters; m is 2, 3, or 4 in models 1, 2, or 3
N	initial population, prior to removal by fishing or trapping
$\hat{N}$	estimate for N
p_i	predicted escapement probability on fishing i ($i = 1,\ldots,k$), or a rough estimate
q_i	predicted capture probability on fishing i ($i = 1,\ldots,k$)
$\hat{q}_i$	estimated capture probability on fishing i ($i = 1,\ldots,k$)

T_i predicted total catch up to and including fishing i $(i = 1,\ldots k)$
$\hat{T}_i$ observed total catch up to and including fishing i $(i = 1,\ldots,k)$
U Hessian matrix of second partial derivatives of $F(\mathbf{Z})$ at $\hat{\mathbf{Z}}$
V approximate covariance matrix for $\hat{\mathbf{Z}}$; inverse of U
$\mathbf{Z}$ model parameter vector, with m components
$\hat{\mathbf{Z}}$ estimated model parameter vector
z_i a component of $\mathbf{Z}$ $(i = 1,\ldots,m)$
ϵ_i normal random error (mean 0, SD 1) in a model for removal data
σ standard deviation related to catch prediction in a model for removal data

Appendix B. Equations (1.2) and (1.4)

This appendix shows that, given (1.1), (1.2) is equivalent to (1.4). First, (1.4) can be proved by mathematical induction from (1.2). The key step comes from writing (1.2) with the index $i + 1$ as follows:

$$(B.1)\quad q_{i+1} = C_{i+1}/(N - C_i - T_{i-1}).$$

Eliminating T_{i-1} from (1.2) and (B.1) gives

$$(B.2)\quad C_{i+1} = q_{i+1}C_i(1 - q_i)/q_i.$$

This result carries (1.4) from the step i to step $i + 1$. Since (1.2) and (1.4) are identical when $i = 1$, the inductive proof of (1.4) is complete.

To prove by induction that (1.2) follows from (1.4), notice that (1.4) implies (B.2), which can also be written in the form

$$(B.3)\quad (C_i/q_i) - C_i = C_{i+1}/q_{i+1}.$$

Assuming (1.2) with the index i, the left side of (B.3) reduces to $N - T_i$; thus, (B.3) becomes

$$(B.4)\quad N - T_i = C_{i+1}/q_{i+1}.$$

This is (1.2) at the index $i + 1$; thus, the proof is complete.

Appendix C. Properties of $F(Z)$

As described in section 2, property 1 is a direct consequence of equation (2.6). To derive (2.6) from the expression (2.4) for L, it is necessary to express the q's in terms of the C's. This can be done using (1.1) and (1.2). After some algebra, it turns out that

$$(C.1)\quad \log [q_i^{\hat{C}_i}(1 - q_i)^{N-\hat{T}_i}] = \hat{C}_i \log (C_i) + (N - \hat{T}_i) \log (N - T_i) - (N - \hat{T}_{i-1}) \log (N - T_{i-1}).$$

When this result is substituted into the expression for $\log (L)$ obtained from (2.4), most of the terms involving $N - T_i$ and $N - T_{i-1}$ cancel. After more algebra, one obtains (2.6).

Property 2 is a variation of an inequality of information theory, which (to my knowledge) has never been proved before. Its proof is facilitated by writing F in the form

$$(C.2)\quad F = F_a + F_b$$

where

$$(C.3)\quad F_a = \sum_{i=1}^{k} \hat{C}_i \log (\hat{C}_i/C_i) + (N - \hat{T}_k) \log [(N - \hat{T}_k)/(N - T_k)]$$

$$(C.4)\quad F_b = N \log (N) - (N - \hat{T}_k) \log (N - \hat{T}_k) - \hat{T}_k \log (\hat{T}_k) - \sum_{j=1}^{\hat{T}_k} \log [j + N - \hat{T}_k)/j].$$

The form (C.2) for F results from adding the final term on the right-hand side of (C.3) to G and subtracting it from H. In (C.4) the sum indexed on j is another expression for the logarithm of the binomial coefficient in H. Its derivation comes from the simple property

$$x! = x\,(x - 1)!$$

for the factorial function

Property 2 will be proved by establishing two subproperties:

Property 2A: $F_a \geq 0$, with equality if and only if $C_i = \hat{C}_i$ for each $i = 1,\ldots,k$.

Property 2B: $F_a \geq 0$, with equality if and only if $N = \hat{T}_k$.

Here, property 2A follows at once from the information inequality (see Rao 1973, p. 58)

$$(C.5)\quad \sum x_i \log (x_i/y_i) \geq 0$$

when

$$(C.6)\quad \sum x_i = \sum y_i,$$

with equality if and only if $x_i = y_i$ for each i. F_a in (C.3) has the form (C.5) with $k+1$ terms, where both the sums (C.6) equal N.

To prove property 2B, notice that the function F_b depends only on the parameter N. Its derivative, after some algebra, turns out to be

$$(C.7)\quad dF_b/dN = \log [N/(N - \hat{T}_k)] - \sum_{j=1}^{\hat{T}_k} (j + N - \hat{T}_k)^{-1}.$$

The first term on the right-hand side of (C.7) is an integral:

$$\log [N/(N - \hat{T}_k)] = \int_{N-\hat{T}_k}^{N} x^{-1}dx.$$

It also happens that the sum on j in (C.7) is a lower rectangular

approximation to this integral; i.e. it is less than the integral. Consequently, the derivative of F_b is positive, and F_b is a strictly increasing function of N. Its smallest value occurs at the smallest admissible value of N, namely $N = \hat{T}_k$, where $F_b = 0$. This proves property 2B and completes the proof of property 2.

Properties 2A and 2B lend insight into the difference between F and the chi-square statistic E. It turns out that F_a and E are approximately equal when the observed and expected catches are close to one another. Also, F_a and E are both zero when $C_i = \hat{C}_i$ for each i. It is the additional component F_b in F that forces F to be positive unless $N = \hat{T}_k$.

Appendix D. Special Results for Models 1 and 2

By combining (1.8) and (2.7), one obtains the function $F(N,q_1)$ for model 1. Differentiating with respect to q_1 gives

$$\text{(D.1)}\quad \frac{\partial F}{\partial q_1} = (kN - \sum_{i=1}^{k} \hat{T}_i)(1 - q_1)^{-1} - \hat{T}_k q_1^{-1}.$$

Similarly, (1.12) and (2.7) combine to give $F(N,q_1,q)$ for model 2. In this case the catchability derivatives turn out to be

$$\text{(D.2)}\quad \frac{\partial F}{\partial q_1} = (q_1 N - \hat{C}_1)(1 - q_1)^{-1} q_1^{-1}$$

$$\text{(D.3)}\quad \frac{\partial F}{\partial q} = [(k-1)N - \sum_{i=2}^{k} \hat{T}_i](1-q)^{-1} - (\hat{T}_k - \hat{C}_1)q^{-1}.$$

Setting the derivative (D.1) equal to 0 and solving for q_1 gives the model 1 estimate (3.2) for q_1. Similarly, (D.2) and (D.3) lead to the model 2 estimates (3.7) and (3.8), respectively.

In model 1, as $N \to \infty$, it follows from (1.10) that

$$\text{(D.4)}\quad C_i \to C_1, \quad i = 1,\ldots,k.$$

Also, since $C_1 = q_1 N$, it follows from (3.2) that

$$\text{(D.5)}\quad C_1 \to \hat{T}_k/k.$$

Thus, all predicted catches converge to the average observed catch per fishing as $N \to \infty$, and

$$\text{(D.6)}\quad T_k \to \hat{T}_k.$$

Substituting this result, as well as Stirling's approximation

$$\log(x!) - 0.5 \log(2\pi x) - x \log(x) + x \to 0$$

as $x \to \infty$, into the function H for model 1 gives

$$\text{(D.7)}\quad H \to \log(\hat{T}_k!) + \hat{T}_k - \hat{T}_k \log(\hat{T}_k)$$

as $N \to \infty$. Finally, combining (D.4)–(D.7) and (2.7) gives the asymptotic result (3.6) for model 1. The result (3.11) for model 2 can be obtained similarly.

Appendix E. Numerical Methods

The major numerical task required here is the process of finding the estimate $\hat{\mathbf{Z}}$ that minimizes $F(\mathbf{Z})$. This was done using Schnute's (1982) implementation of the Apple II microcomputer of the simplex search method, described originally by Nelder and Mead (1965). To take advantage of Schnute's software, all that is needed is a computer program to compute $F(\mathbf{Z})$. The program used here allows the user to select one of eight options for F, as summarized below:

Model 1	Model 2	Model 3
(1) $F(N,q_1)$	(4) $F(N,q_1,q)$	(7) $F(N,q,q,a)$
(2) $F(N,C_1)$	(5) $F(N,C_1,C_2)$	(8) $F(N,C_1,C_2,C_3)$
(3) $F_1(N)$	(6) $F_2(N)$	

When options (3) or (6) are selected, the program also allows the user to specify a value f for F and choose the function

$$S(N) = [F(N) - f]^2$$

as the function to be minimized. In this case the minimum of S is 0, achieved at the value of N for which F equals f. This feature allows the program to be used in finding the end points of a confidence interval for N determined from (3.5) or (3.10).

The simplex search method, together with options (3) and (6), gives all the analysis in Tables 2 and 3, except the standard deviations in Table 3. For the latter, the matrix $\boldsymbol{U}$ of second partial derivatives of F was computed numerically by Newton's divided difference method, using options (1), (2), (4), and (5). As described in section 5, the analysis based on options (2) and (5) is reported in Table 3, rather than based on (1) and (4). The software to manage the difference calculation is an extension to the package by Schnute (1982); it uses the same module for computing F as the simplex search algorithm. In contrast with Moran (1951), Zippin (1956), and Otis et al. (1978), this method avoids the use of analytical derivatives and Stirling's approximation in the Hessian calculation.

The calculation of F involves a subroutine to compute $\log(x!)$. For readers without access to such a subroutine, the following can be used. Define

$$\text{(E.1)}\quad g(y) = y \log(y) - y + 0.5 \log(2\pi/y) + \sum_{i=1}^{5} g_i y^{1-2i}$$

where the constants $g_1,\ldots,g_5$ are $1/12$, $-1/360$, $1/1260$, $-1/1680$, and $1/990$, respectively. Then

$$\text{(E.2)}\quad \log(x!) = \begin{cases} g(x+1), & x \geq 3 \\ g(x+4) - \log[(x+1)(x+2) \times (x+3)], & 0 \leq x \leq 3. \end{cases}$$

If ϵ represents the error in (E.2), then

$$0 > \epsilon > -5 \times 10^{-10}$$

so that (E.2) slightly overestimates $\log(x!)$. This approximation is based on an asymptotic expansion of the gamma

function in terms of Bernoulli numbers (Abramowitz and Stegun 1964, p. 257). Earlier work based on Stirling's approximation omits the final sum of five terms in (E.1). These can be significant when x is small, as happens here in computing $\log [(N - \hat{T}_k)!]$ when N is close to the observed total catch.

A Stochastic Age-Structured Population Model of Striped Bass (*Morone saxatilis*) in the Potomac River[1]

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Deterministic age-structured models of fish populations neglect apparently stochastic fluctuations in the catch per unit effort of yearlings and of adult fish. We describe a model of an age-structured population in which the survival of eggs to yearlings fluctuates randomly, but all other age-specific rates of survival and of egg-laying are constant. For such a stochastic model, two measures of the long-term population growth rate are the average growth rate of the population size and the growth rate of the average population size. We compute both measures analytically for a simplified model representing only eggs and reproductive adults. For a model of the striped bass (*Morone saxatilis*) population spawning in the Potomac River, we compute both point and interval estimates of the growth rate of the average population size. We illustrate some statistical tests of the correctness of our stochastic model.

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Les modèles déterministes, structurés par âge, de populations de poissons négligent les fluctuations apparemment stochastiques des prises par unité d'effort de poissons de l'année et de poissons adultes. Nous décrivons dans l'article qui suit une population structurée par âge dans laquelle la survie des oeufs jusqu'à l'âge d'un an fluctue de façon aléatoire, alors que tous les autres taux particuliers à l'âge de survie et de déposition des oeufs sont constants. Dans un modèle stochastique de ce genre, le taux de croissance moyen de l'effectif de la population et le taux de croissance de l'effectif moyen de la population sont deux mesures de la croissance à long terme de cette population. Nous calculons les deux mesures analytiquement, dans un modèle simplifié représentant seulement les oeufs et les adultes reproducteurs. Dans le cas d'un modèle de population de bars rayés (*Morone saxatilis*) frayant dans le Potomac, nous produisons des estimations à la fois ponctuelles et par intervalles du taux de croissance de l'effectif moyen de la population. Nous donnons quelques exemples de tests statistiques de notre modèle stochastique.

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To measure the actual and potential impact of power plant operations on fish populations, one must construct mathematical models of the fish populations. Deterministic age-structured models that employ the Leslie matrix (Keyfitz 1968) neglect apparently stochastic fluctuations in fish populations. Fisheries biologists have generalized the Leslie matrix by incorporating stochastic fluctuations in one or more age-specific vital rates. The behavior of these models has been studied primarily by numerical computation (Allen and Basasibwaki 1974; Jensen 1975; Vaughan 1977a, 1977b; DeAngelis et al. 1977; Christensen et al. 1982; Deriso 1980; Ginzburg et al. 1983; D. Ludwig and C. J. Walters, unpublished data).

Independently of these developments among students of fish populations, students of mallard (*Anas platyrhynchos*) populations (Anderson 1975) and of human populations (LeBras 1974; Cohen 1976) investigated the effects on popu-

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lation growth of random fluctuations in age-specific vital rates. Analytical approaches to computing long-run growth rates have been developed (Cohen 1977a, 1977b, 1979a, 1979b, 1979c, 1980; Lange 1979; Lange and Hargrove 1980; Lange and Holmes 1981; Tuljapurkar and Orzack 1980).

Our first purpose was to analyze a model developed by fisheries biologists, using the mathematical methods just cited, and then compute long-run growth rates of fish populations subject to random fluctuations in the survival of eggs. Our second purpose was to show how to approximate the sampling variability in the estimate of the long-run growth rate of average population size. Our third purpose was to illustrate statistical methods for testing whether the model is appropriate to real data of the rather limited, and less than scientifically ideal, kind that are likely to be available in practical situations in which many managerial and regulatory decisions are required.

We use data on the population of striped bass (*Morone saxatilis*) spawning in the Potomac River. We anticipate that these results will aid evaluations of the aquatic impact of power plants by making it possible to calculate long-run effects on growth rates of fish populations.

Age-Structured Model with Random Survival of Eggs

We consider a female population of fish at yearly intervals just after the spawning of eggs in early spring. We take the unit of time to be 1 yr. We let $t = 0$ be the time of initial observation, $y_0(t)$ the number of the newly spawned female eggs at time t, and $y_i(t)$ the number of i-yr-old female fish at the end of the spawning season in year t, where $i = 1, 2, \ldots, k$ and k is the maximum age of fish considered. We let $\mathbf{y}(t)$ be the column vector with elements $y_0(t)$, $y_1(t)$, $\ldots$, $y_k(t)$. We shall call $\mathbf{y}(t)$ the age census at time t.

It is realistic to attribute the eggs spawned in year t to the female population alive in year t. Assuming no density dependence in the laying of eggs, we suppose, with $F_i = 0$ for all nonreproductive age-classes, that

$$y_0(t) = \sum_{i=1}^{k} F_i y_i(t)$$

where F_i is the average number of female eggs laid in year t per female fish aged i in that year and $F_k > 0$. Define $F_0 = 0$, since eggs lay no eggs. The post-egg female population at time t (neglecting immigration) consists of the survivors of the female population (including eggs) at time $t - 1$:

$$y_i(t) = s_{i-1}y_{i-1}(t-1), \; i = 1, \ldots, k$$

where $s_i > 0$ is the fraction of female individuals (eggs or fish) aged i at $t - 1$ who survive in the population (neither dying nor emigrating) to age $i + 1$ at time t.

In conventional Leslie matrix models, this year's births (eggs) are usually attributed to last year's female population, rather than to this year's, as in the first equation above. To express $y_0(t)$ in terms of the post-egg female population at time $t - 1$, we define $s_k = 0$, $F_{k+1} = 1$ (we could pick any finite value for F_{k+1}), and the effective fecundity of age class i by

$$E_i = F_{i+1}s_i, \; i = 0, \ldots, k.$$

Then upon substitution, since $E_k = 0$,

$$\begin{aligned} y_0(t) &= \sum_{i=1}^{k} F_i s_{i-1} y_{i-1}(t-1) + E_k y_k(t-1) \\ &= \sum_{j=0}^{k} E_j y_j(t-1). \end{aligned}$$

Thus, changes over time in the age census can be described in the conventional way by

$$\mathbf{y}(t) = \mathbf{L}(t)\mathbf{y}(t-1) \tag{1}$$

where $\mathbf{L}(t)$ is a $(k + 1) \times (k + 1)$ Leslie matrix:

$$\mathbf{L}(t) = \begin{bmatrix} E_0 & E_1 & E_2 & \ldots & E_{k-1} & E_k \\ s_0(t) & 0 & 0 & \ldots & 0 & 0 \\ 0 & s_1 & 0 & \ldots & 0 & 0 \\ 0 & 0 & s_2 & \ldots & 0 & 0 \\ \cdot & \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & \cdot & & \cdot & \cdot \\ 0 & 0 & 0 & \ldots & s_{k-1} & 0 \end{bmatrix} \tag{2}$$

We assume in (2) that all elements of the Leslie matrix are constant over time except $s_0(t)$, the survival of eggs at time $t - 1$ to age class 1 at time t. For $E_0 = F_1 s_0(t)$ to be independent of t, we must have $E_0 = F_1 = 0$. The assumption that all elements of $\mathbf{L}(t)$ are constant except $s_0(t)$ is a simplification as a first step in model building. Although we recognize that other elements of $\mathbf{L}(t)$ vary, we believe that the overwhelming source of variation lies in the survival of eggs (Hunter 1976). In particular, we assume that the variation in the survival of eggs is large compared even with the variation in adult mortality due to changes in fishing over time. If this last assumption is false, the effect on our results is outlined briefly below, at the end of the summary of our data analytic procedure and results, and in greater detail by C. P. Goodyear, J. E. Cohen, and S. W. Christensen (unpublished data). The present model and methods should not be used without modification in situations in which this assumption is known to be false.

This model is relevant to cases other than fish populations. Among white-crowned sparrows (*Zonotrichia leucophrys nuttali*) in coastal California, mortality varies mainly during the period from fledging to January 1 and not significantly thereafter (Baker et al. 1981, p. 643). If annual age classes were used for these birds, mortality variation would be concentrated in survival to age 1, as in the model (2).

As is standard in probability theory, we shall use the symbol $E()$ to denote the average, mean, or expected value of whatever random variable is enclosed in parentheses.

To complete the description of the model, we specify that the average survival $E(s_0(t))$ of eggs is identical from year to year and that the survival in any one year is independent of the survival in any other year. Thus, the random variables $s_0(t)$ are independent and $E(s_0(t))$ is independent of t. These strong assumptions can be tested.

If the weather at the time of spawning is the principal influence on the survival of eggs (Ulanowicz and Polgar 1980), it may be reasonable to guess that the weather at that season is independent from year to year. However, analytical methods exist for analyzing models in which the distribution of Leslie matrix elements in one year does depend on the Leslie matrix elements that occurred the previous year. For the present model of independence from year to year, we shall be applying only a special case of those methods.

How should the long-run growth rate of a population with stochastic vital rates be measured? When the Leslie matrix is constant over time, so that $\boldsymbol{L}(t) = \boldsymbol{L}$, there is only one answer (Keyfitz 1968): the intrinsic rate of natural increase r of the population is the natural logarithm of the dominant eigenvalue of $\boldsymbol{L}$. (Henceforth, we shall always use log and logarithm to mean natural logarithm.) Following is the reason for this choice when $\boldsymbol{L}(t) = \boldsymbol{L}$. For large times t, nonzero elements of $\boldsymbol{L}^t$ are proportional to the tth power of the dominant eigenvalue of $\boldsymbol{L}$, which is the same as e^{rt}, and therefore so are all elements of the age census $\boldsymbol{y}(t)$. If $Y(t)$ is the total population size at time t, then for large values of t, $Y(t)$ is proportional to e^{rt}. Using c for the constant of proportionality, we have, when the Leslie matrix is constant,

$$(3) \quad Y(t)/e^{rt} = c \text{ for large } t.$$

Taking logarithms of both sides of (3), dividing by t, and letting t become large gives

$$(4) \quad r = \lim_{t\to\infty} (1/t) \log Y(t).$$

Since the asymptotic rate of growth is the same for all age classes in both the deterministic measure (4) and the stochastic measures (5) and (6), it makes no difference here whether nonreproductive age classes are included in $Y(t)$ or excluded.

When $\boldsymbol{L}(t)$ varies randomly, so does $Y(t)$, and there is no unique "intrinsic rate of natural increase." Instead, there are several possible generalizations of (4). It becomes necessary to choose some overall measure of the behavior of $\lim_{t\to\infty} (1/t) \log Y(t)$. One possibility is to take the median of $\lim_{t\to\infty} (1/t) \log Y(t)$ as a measure of population growth rate. Two other possibilities that have been studied analytically, and which we shall now describe in more detail, are to compute the average of the growth rate of population size and the growth rate of the average population size. It is important to realize that these two are different measures of population growth rate. Both measures of population growth rate have been studied in the context of fishing yield by Vaughan (1977a).

The average of the growth rate of population size is, by definition,

$$(5) \quad \log \lambda = \lim_{t\to\infty} E[(1/t) \log Y(t)].$$

The growth rate of the average population size is, by definition,

$$(6) \quad \log \mu = \lim_{t\to\infty} (1/t) \log E[Y(t)].$$

The only difference between these two quantities is the position of $E()$. In $\log \lambda$, the growth rate of each separate realization of the population is computed first (think of each realization as one computer simulation, or as one of a large number of demes), and then the growth rates are averaged. In $\log \mu$, the average population size at each time t is computed (averaged over all simulations or over all demes), and then the growth rate of the average is found. If the Leslie matrices $\boldsymbol{L}(t)$ do not vary at all, so that $s_0(t)$ is actually constant, then the three measures, the median of $\lim_{t\to\infty} (1/t) \log Y(t)$, $\log \lambda$, and $\log \mu$, are all identical to r.

In general, the exact computation of $\log \lambda$, even for independently distributed Leslie matrices with constant mean, is quite difficult (Cohen 1977a, 1979a), although we will find $\log \lambda$ easily in a simple example in the next section. The computation of $\log \mu$, on the other hand, is generally easy, as we now show. Equation (1) implies that, given $\boldsymbol{y}(0)$,

$$(7) \quad \boldsymbol{y}(t) = \boldsymbol{L}(t)\boldsymbol{L}(t-1) \ldots \boldsymbol{L}(2)\boldsymbol{L}(1)\boldsymbol{y}(0).$$

Take the average of both sides:

$$(8) \quad E[\boldsymbol{y}(t)] = E[\boldsymbol{L}(t)\boldsymbol{L}(t-1) \ldots \boldsymbol{L}(2)\boldsymbol{L}(1)]\boldsymbol{y}(0).$$

Since successive Leslie matrices are independent, the expectation of the product is the product of the expectations; and since the mean of $\boldsymbol{L}(t)$ is identical over time,

$$(9) \quad E(\boldsymbol{L}(t)\boldsymbol{L}(t-1) \ldots \boldsymbol{L}(1)) = (E(\boldsymbol{L}(1)))^t.$$

Substituting (9) into (8) gives

$$(10) \quad E[\boldsymbol{y}(t)] = (E(\boldsymbol{L}(1)))^t \boldsymbol{y}(0).$$

Assuming that $E(s_0(t)) > 0$, so that on the average at least some eggs survive to age 1, and provided that the initial population is positive in all age classes, $E[Y(t)]$ grows proportionally to ρ^t for large t, where ρ is the dominant eigenvalue of the average Leslie matrix $E(\boldsymbol{L}(1))$. Consequently, for some constant of proportionality c,

$$(11) \quad E[Y(t)]/(\rho^t) \to c \text{ for large } t.$$

Taking logarithms of both sides of (11), dividing by t, and letting t become large gives $\log \rho = \lim (1/t) \log E[Y(t)]$. Comparison with (6) shows that ρ must equal μ, i.e. for this model, μ is exactly the dominant eigenvalue of the average Leslie matrix $E(\boldsymbol{L}(1))$.

The actual procedure to compute (6) is simple: when successive Leslie matrices are independently distributed with constant mean, take the average of the Leslie matrices (this means average each element of the matrix one at a time), find the largest eigenvalue of the matrix (using standard computer programs), take the natural logarithm of the eigenvalue, and the growth rate of the expected population size is found. Error estimates for μ are derived below.

Since the logarithm is a concave function, i.e. its graph has decreasing slope,

$$(12) \quad \log \lambda \leq \log \mu$$

and the computation of (6) gives an upper bound for the value

of (5). This inequality (12) holds for any stochastic population model, not merely those analyzed here.

Simple Example: One Reproductive Age Class

To illustrate the computation of $\log \lambda$ and $\log \mu$ in a simple example, let us approximate the structure of the fish population by two age classes, so that we have only nonreproductive eggs and reproductive adults:

$$(13)\quad \boldsymbol{L}(t) = \begin{pmatrix} 0 & E_1 \\ s_0(t) & 0 \end{pmatrix}.$$

(Here, we temporarily abandon the assumption, made in the previous section, that the effective fertility of the oldest age class, which is E_1 in (13), is 0. If we did take $E_1 = 0$, the population would go extinct after 2 yr. But taking $E_1 > 0$, as we shall do in this section only, means that this year's eggs are produced by last year's adults, not by this year's adults. We accept this modification of the model described in the previous section in order to illustrate some calculations in a simple case.)

First, we shall compute $\log \lambda$. Notice that

$$(14)\quad \boldsymbol{L}(2t)\boldsymbol{L}(2t-1) = \begin{pmatrix} E_1 s_0(2t-1) & 0 \\ 0 & E_1 s_0(2t) \end{pmatrix}, t = 1, 2, \ldots$$

which is nice because it is very easy to multiply diagonal matrices (a diagonal matrix has zero elements everywhere except possibly on the main diagonal): one simply multiplies corresponding diagonal elements. So we get, using (14) in (7),

$$(15)\quad \begin{aligned} y_0(2t) &= E_1^t s_0(1)s_0(3) \ldots s_0(2t-1)y_0(0) \\ y_1(2t) &= E_1^t s_0(2)s_0(4) \ldots s_0(2t)y_1(0). \end{aligned}$$

Hence

$$(16)\quad \begin{aligned} (1/(2t))\log y_0(2t) &= (1/2)\log E_1 \\ &\quad + (1/(2t))\Big[\sum_{u=1}^{t} \log s_0(2u-1) + \log y_0(0)\Big] \\ (1/(2t))\log y_1(2t) &= (1/2)\log E_1 \\ &\quad + (1/(2t))\Big[\sum_{u=1}^{t} \log s_0(2u) + \log y_1(0)\Big]. \end{aligned}$$

For all μ, $\log s_0(2u)$ and $\log s_0(2u-1)$ are observed or sampled values of the same random variable $\log s_0(t)$, which we shall assign the same probability distribution as $\log s_0(1)$. Now we assume that $E[\log s_0(1)]$ exists. Then, the strong law of large numbers applies to the right sides of (16) and implies that, with probability 1,

$$(17)\quad \begin{aligned} \lim_{t\to\infty} [2t]^{-1} \log y_0(2t) &= \lim_{t\to\infty} [2t]^{-1} \log y_1(2t) \\ &= (1/2)\log E_1 \\ &\quad + (1/2)\, E(\log s_0(1)). \end{aligned}$$

Since the almost sure limit is the same for $y_0(t)$ and $y_1(t)$, it is the same for their sum. Consequently, the mean and median growth rates of the total population size (eggs plus adults) for large t are also the same:

$$(18)\quad \log \lambda = (1/2)\log E_1 + (1/2)\, E(\log s_0(1)).$$

This expression (18) for $\log \lambda$ is valid if $s_0(t) > 0$ are independently and identically distributed and if $E(\log s_0(1))$ exists.

Now assume $s_0(t)$ is approximately lognormally distributed, specifically,

$$(19)\quad s_0(t) = se^{mz(t)},\ t = 1, 2, \ldots$$

where s is a positive constant and $z(t)$ is a normally distributed random variable with mean 0 and variance 1 and independent from one time to another. Thus, $mz(t)$ has mean 0 and variance m^2. This model (19) must be only an approximation because, when $m > 0$, $mz(t)$ has a positive probability of being larger than any fixed bound, so that $se^{mz(t)}$ has a positive probability of being larger than 1. This makes no sense if $s_0(t)$ is to be interpreted as a survival proportion. We shall assume now (and later will actually observe) that the probability that $s_0(t)$ exceeds 1 is small enough to be neglected.

If $m = 0$, $\boldsymbol{L}(t)$ is constant with dominant eigenvalue $(E_1 s)^{1/2}$ and we recover the deterministic intrinsic rate $\log \lambda = r = (1/2)\log(E_1 s)$. If $m \neq 0$, we obtain, by substituting (19) into (18),

$$(20)\quad \log \lambda = (1/2)\log(E_1 s) + (1/2)\, E(mz(1)) = r,$$

regardless of the value of m, because $E(mz(1)) = 0$. Thus, in this example, regardless of the variance of the randomly varying exponent in the survival of eggs, the mean growth rate of population size is identical to that of the deterministic population model of the same structure but with no variance in the survival of eggs.

Now we compute $\log \mu$. We shall use the standard facts that (Aitchison and Brown 1957)

$$(21)\quad E(e^{mz(t)}) = e^{(1/2)m^2}$$

and that the expectation of a product of independent random variables is the product of the expectations. Taking the average of both sides in (15) gives

$$(22)\quad \begin{aligned} E[y_0(2t)] &= E_1^t s^t e^{tm^2/2}\, y_0(0) \\ E[y_1(2t)] &= E_1^t s^t e^{tm^2/2}\, y_1(0) \end{aligned}$$

so that

$$(23)\quad \log \mu = (1/2)\log(E_1 s) + m^2/4.$$

Comparing (23) with (20) confirms the general inequality $\log \mu \geq \log \lambda$. In addition, we see that $\log \mu$ is a linearly increasing function of the variance of the exponent $mz(t)$ in the survival of eggs. In this example, when there is variation in the survival of eggs, the growth rate in the mean population size always exceeds the intrinsic rate of natural increase in the deterministic model of the same structure. However, if the

variation in the survival of eggs becomes too large, the specific model (19) becomes inappropriate because it permits $s_0(t)$ to exceed 1 with nonnegligible probability.

As a check on the correctness of (23), we may apply the procedure described at the end of the previous section, without the necessity of relying on the explicit formula (15). The procedure says first to find the expected Leslie matrix:

$$(24)\quad E(\boldsymbol{L}(t)) = \begin{pmatrix} 0 & E_1 \\ se^{m^2/2} & 0 \end{pmatrix}.$$

The largest eigenvalue is $(E_1 s)^{1/2}\, e^{m^2/4}$, and the logarithm of the eigenvalue is identical to (23).

Although this simple example is artificial, because it considers only one adult age class, it illustrates the meaning of $\log \lambda$ and $\log \mu$. It also illustrates the relations between these measures of growth and the intrinsic rate of natural increase of a deterministic model.

Under the conditions of this example, the median value of $\lim_{t\to\infty} (1/t) \log Y(t)$ is identical to $\log \lambda$; in fact, all realizations except for a set of probability 0 have growth rates given by $\log \lambda$. More general conditions under which $\log \lambda$ is the growth rate of almost all realizations of a product of random matrices are given by the pioneering paper of Furstenberg and Kesten (1960).

Approximate Standard Deviation of the Estimate of a Growth Rate

We now return to the general model (1) and (2). Suppose that all the elements in the Leslie matrix (2), except $s_0(t)$, are known either exactly or with negligible uncertainty. Suppose $s_0(1), s_0(2), \ldots, s_0(N)$ are a sample of N observed values of $s_0(t)$. Let the sample mean be $\bar{s}_0$ and the variance of the sample mean be $\mathrm{Var}(\bar{s}_0)$. (Recall that the variance of the sample mean is the sample variance divided by N.) Here, we assume that $\mathrm{Var}(s_0(t))$ is independent of t in addition to assuming that $E(s_0(t))$ is independent of t.

The sample mean Leslie matrix $\bar{\boldsymbol{L}}$ is obtained by replacing $s_0(t)$ in (2) with $\bar{s}_0$. Let $\hat{\mu}$ be the dominant eigenvalue of $\bar{\boldsymbol{L}}$. This $\hat{\mu}$ is the sample estimate of the dominant eigenvalue μ of the expected Leslie matrix $E(\boldsymbol{L}(t))$.

The purpose of this section is to derive an approximation to the variance of $\log \hat{\mu}$. Notice that the dominant eigenvalue μ is a constant, not a random variable; variation in $\hat{\mu}$ arises from the sampling variability of $\bar{s}_0$ around its true mean $E(s_0(t))$.

Our approximation to $\mathrm{Var}(\log \hat{\mu})$ rests on the crude but commonly used Taylor series technique, and neglects the uncertainty in all other elements of $\boldsymbol{L}(t)$.

Let f be the function that produces $\log \mu$, given $E(s_0(t))$, i.e.

$$(25)\quad \log \mu = f(E(s_0(t))).$$

This function is simply the log of the dominant eigenvalue of the matrix $\boldsymbol{L}(t)$ with $s_0(t)$ replaced by $E(s_0(t))$. Thus

$$(26)\quad \log \hat{\mu} = f(\bar{s}_0).$$

Now, expand $f(\bar{s}_0)$ in a Taylor series about $f(E(s_0(t)))$ and drop all but the first two terms:

$$(27)\quad f(\bar{s}_0) = f(E(s_0(t))) + [\bar{s}_0 - E(s_0(t))](df/ds_0)_{s_0=\bar{s}_0}.$$

Then subtract (25) from (26) and approximate $f(\bar{s}_0)$ by its truncated Taylor series expression (27):

$$(28)\quad \log \hat{\mu} - \log \mu = [\bar{s}_0 - E(s_0(t))](df/ds_0)_{s_0=\bar{s}_0}.$$

Square both sides of (28) and take expected values. Then assume $E(f(\bar{s}_0))$ is close to $f(E(s_0(t)))$, which will asymptotically be true for large numbers of data points. We obtain

$$(29)\quad \mathrm{Var}(\log \hat{\mu}) \sim E[(\log \hat{\mu} - \log \mu)^2] = \mathrm{Var}(\bar{s}_0)[(df/ds_0)_{s_0=\bar{s}_0}]^2.$$

To compute df/ds_0, we can use formula (11) of Caswell (1978, p. 218):

$$(30)\quad (df/ds_0)_{s_0=\bar{s}_0} = (d \log \mu/d\mu)(d\mu/ds_0)_{\mu=\hat{\mu},\, s_0=\bar{s}_0} = u_1 v_0/\hat{\mu}$$

where u is the left row eigenvector and v is the right column eigenvector corresponding to the dominant eigenvalue $\hat{\mu}$ of $\bar{\boldsymbol{L}}$ and $(u, v) = 1$. The particular elements u_1 and v_0 appear because of the position of s_0 in $\boldsymbol{L}$. The easiest numerical method to compute the dominant eigenvalue of a Leslie matrix, namely the power method, produces u and v with no extra effort.

We shall illustrate the use of (29) below.

Potomac River Striped Bass

In this section, we shall use data on the striped bass population that spawns in the Potomac River to test the model (1) and (2) and to illustrate numerically how to estimate $\log \mu$, the long-run rate of growth of the average population size.

A major assumption of the model (1) is that mortality is density independent. While this assumption is open to controversy in general, two lines of evidence make it plausible for the Chesapeake striped bass populations, including the population spawning in the Potomac River. First, in an analysis of the striped bass landings, Van Winkle et al. (1979) found a significant periodicity of 6 yr. This period length is not consistent with the period length that would be expected if the stock density were the causative agent (Van Winkle et al. 1979; Goodyear 1980). In addition, studies of the influence of environmental variables indicate that a large part of the annual variations in year-class strength are caused by variations in environmental factors, principally freshwater discharge and water temperature (Kohlenstein 1980; Ulanowicz and Polgar 1980). These observations indicate that the level of mortality is, at best, only weakly related to the size of the stock. We now proceed to a detailed analysis of data.

The Data

We estimated F_i, the number of female eggs per female fish of age i for striped bass in the Chesapeake Bay, for $i = 1, 2, \ldots, 15$, as shown in Table 1. These estimates are rounded to reflect the substantial uncertainty associated with them. The qualitative shape of the relation between age and female

TABLE 1. Estimated age-specific fecundity F_i of striped bass.

Age (i)[a]	Fecundity (F_i)[b]
1	0
2	0
3	0
4	33 000
5	170 000
6	350 000
7	530 000
8	680 000
9	900 000
10	1 000 000
11	1 200 000
12	1 500 000
13	1 800 000
14	2 100 000
15	2 400 000

[a]Age is measured in years since spawning.

[b]F_i estimates the average fertile female eggs laid during the spawning season per female fish of age i as a function of age at maturity estimated by Merriman (1941) and estimated fecundity of mature females. Fecundity of mature females was estimated by converting lengths at annulus formation for females to weight (Mansueti 1961) and calculating the number of eggs using the weight−fecundity relationship reported by Jackson and Tiller (1952). The number of mature ova was then divided by 2 on the assumption that half would be females upon fertilization. Fecundities of ages 13−15 were estimated as the sum of the fecundity of the previous age plus the increment between the fecundity of age 11 and that of age 12.

eggs per fish is believed to be correct. We assume these data apply to the striped bass spawning in the Potomac River.

Since the fecundity estimates are uncertain, possible fluctuations in fecundity from year to year can be neither excluded nor measured. If present, they would contribute to variability in the number of eggs spawned via (32) below and hence via (33) to the estimated variability in the survival proportion of eggs. Thus, possible variations in fecundity may contribute to the estimated variability in the survival of eggs. In terms of the model (1) and (2), we take the number of post-egg age-classes to be $k = 15$.

Precise data on the age-specific survival proportions s_i are lacking. Different investigators report a substantial range of estimates of post-egg survival. We consider three sets of values for s_i, namely, for $i = 1, \ldots, 14$, $s_i = 0.4$; or $s_i = 0.5$; or $s_i = 0.6$. These three cases are chosen to cover most of the range of uncertainty about the true survival proportions. It will appear that the inferences to be drawn are insensitive to the value chosen for s_i. Calculations not presented here show that these inferences are also insensitive to minor refinements in the age distribution of mortality, e.g. to allow for a reported slightly increased mortality at ages 3−6 (Kohlenstein 1980; Polgar 1980). If, contrary to our assumption, the s_i are not constant in time, their fluctuations may contribute to the estimated variability in the survival of eggs in the same way that fluctuations in fecundity may, via (32) and (33).

Since $E_i = s_i F_{i+1}$, these assumptions plus Table 1 determine all the elements of the Leslie matrix $L(t)$ in (2) except $s_0(t)$.

To estimate the distribution of the random variable $s_0(t)$, we exploit the time series reported by Florence (1980) and supplemented subsequently by the Maryland Department of Natural Resources. Table 2 shows the average catch (number of individual male and female fish) per beach seine haul of fingerling striped bass in the Potomac River, based on a standardized seining procedure conducted in late summer and early autumn of each year by the Maryland Department of Natural Resources. We have not been able to determine the standard deviation, due to sampling variability, that should be associated with each of these average annual values. It is therefore not possible for us, at this time, to determine what biological significance should be attached to the differences between years in average catches. Thus, the following calculations should tentatively be treated as illustrative rather than as definitive.

It is possible that the Maryland Department of Natural

TABLE 2. Average catch (number of individuals of both sexes) per beach seine haul of fingerling striped bass in the Potomac River breeding site of Chesapeake Bay, based on annual surveys conducted by the Maryland Department of Natural Resources.

		Survival of eggs assuming adult survival s_i is:[b]		
Year	Young-of-year[a]	0.4	0.5	0.6
1954	5.2			
1955	5.7			
1956	6.2			
1957	2.6			
1958	8.4			
1959	1.6			
1960	4.3			
1961	25.7			
1962	19.7			
1963	1.1			
1964	29.2			
1965	3.4			
1966	10.5			
1967	1.9			
1968	0.7			
1969	0.2			
1970	20.1	9.34×10^{-7}	2.98×10^{-7}	1.06×10^{-7}
1971	8.5	1.10×10^{-4}	3.23×10^{-5}	1.07×10^{-5}
1972	1.8	5.83×10^{-5}	1.59×10^{-5}	4.92×10^{-6}
1973	2.1	1.86×10^{-5}	4.50×10^{-6}	1.26×10^{-6}
1974	1.5	3.58×10^{-5}	7.39×10^{-6}	1.80×10^{-6}
1975	7.7	2.04×10^{-5}	5.74×10^{-6}	1.52×10^{-6}
1976	3.2	6.33×10^{-5}	2.13×10^{-5}	6.82×10^{-6}
1977	1.9	2.65×10^{-5}	8.32×10^{-6}	2.69×10^{-6}
1978	7.9	2.11×10^{-5}	5.84×10^{-6}	1.78×10^{-6}
1979	2.1	1.29×10^{-4}	3.21×10^{-5}	9.03×10^{-6}
1980	2.3	3.73×10^{-5}	9.82×10^{-6}	2.70×10^{-6}
1981	1.4	3.66×10^{-5}	1.08×10^{-5}	3.29×10^{-6}
1982	—	2.45×10^{-5}	7.05×10^{-6}	2.13×10^{-6}

[a]Young-of-year t is taken as an index of $y_1(t+1)$, the number of 1-yr-old female fish at the time of the spawning season in calendar year $t+1$. Thus, the average catch of 2.3 individuals in 1980 indexes the 1-yr-old female population in 1981. (Data courtesy of Maryland Department of Natural Resources (B. Florence).)

[b]Survival of eggs $s_0(t)$ from year $t-1$ to year t is $y_1(t)/y_0(t-1)$, where $y_0(t-1)$, the number of eggs spawned in $t-1$, is calculated assuming that the post-egg annual survival of females is s_i, $i = 1, 2, \ldots, 14$.

Resources will release more detailed data in the future. The need to make intelligent practical use of data such as those in Table 2 seems to us to justify the presentation of useful methods now.

Fingerlings are approximately 3–5 mo old and are assumed to have a sex ratio of approximately 1:1. We shall assume that the mortality between fingerling age and 1 yr of age is constant and therefore shall take the average number of fingerlings sampled in calendar year t as an index of the number $y_1(t+1)$ of 1-yr-old female striped bass in the population in the following spring of year $t+1$, $t = 1954, \ldots, 1981$. The total number of fingerlings differs from the number of female yearlings only by a constant of proportionality, so no error will be introduced in the computation of $s_0(t)$ (see (33) below).

Constructing the Spawning Stock and Survival Proportion of Eggs

We now use this time series of $y_1(t)$ in Table 2 plus the assumed post-egg survival coefficients to estimate the age structure of the post-egg population (i.e. the population aged 1–15 yr) for the years 1969–81. In 1969, the 1-yr-olds of 1955 (i.e. the fingerlings caught in 1954) who have survived will be 15, since $1 + (1969-1955) = 15$. Therefore, the expected number of 15-yr-olds in 1969 is $y_{15}(1969) = y_1(1955)s_1s_2 \ldots s_{14}$. Similarly, the expected number of 14-yr-olds in 1969 is the number of 1-yr-olds in 1956 who have survived 13 yr, i.e. $y_{14}(1969) = y_1(1956)s_1 \ldots s_{13}$. In general, for $t = 1969, \ldots, 1980$, the post-egg age structure is given by the data for 1-yr-olds and this computation for older fish:

$$(31)\quad y_i(t) = y_1(t-i+1)s_1s_2 \ldots s_{i-1},\ i = 2, \ldots, k.$$

We do not know the entire age structure prior to 1969 because we do not know the fingerling age-classes in years prior to 1954. Since the four oldest age-classes contribute approximately 3% to the net rate of reproduction of the population, one could approximate those four age-classes by a single age-class, without much loss in accuracy, in order to extend the time series of post-egg censuses for a few additional years. Computations not reported here suggest that combining the last few age-classes has little effect on the inferences that follow.

Given the post-egg age structure for $t = 1969-81$, we compute the expected number of eggs spawned in 1969 to 1981 by

$$(32)\quad y_0(t) = \sum_{i=1}^{k} F_i y_i(t),\ t = 1969, \ldots, 1981.$$

Then we compute the proportion of eggs surviving to 1 yr of age by the relation, which is a consequence of (1) and (2),

$$(33)\quad s_0(t) = y_1(t)/y_0(t-1),\ t = 1970, \ldots, 1982.$$

This computation is independent of the form of the probability distribution assumed for $s_0(t)$.

Although we started with a time series of data for fingerlings from 1954 to 1981, we are left with an estimate of the 1-yr survival of eggs only for the years 1970–82 because of the long life and sustained fecundity of the striped bass. Figure 1 plots $\log s_0(t)$ as a function of year t for each of the assumed values of s_i.

In linear regressions not reported here, we have investigated whether the proportion of eggs surviving, estimated from (33), appears to depend on the expected number of eggs spawned, estimated from (32). We found no significant evidence of density dependence in the survival of eggs.

Is the Survival Proportion of Eggs Independently Distributed with Constant Mean?

We now test the assumption that $s_0(t)$ is distributed independently over time with constant mean. It is crucial to test this assumption before estimating $\log \mu$ because the correctness of our method of estimation depends on this assumption. If $s_0(t)$ is not independently distributed over time, the estimate of $\log \mu$ obtained under the assumption of independence may be too high or too low. The more nearly independent $s_0(t)$, the better the estimation procedure that assumes independence.

The first test is visual inspection of Fig. 1. There appears to be no clear increasing or decreasing trend of $\log s_0(t)$ as a function of t, for any value of s_i. There also appear to be no excessively long runs of data points above or below the regression line, nor any clear alternation above and below. The only possible suggestion of change over time may be in the variance of $s_0(t)$, which might decrease as time increases. We now test these observations more formally.

In the linear regression $\log s_0(t) = a + bt$, plotted in Fig. 1, the coefficient b and its standard deviation are 0.095 ± 0.089, 0.095 ± 0.087, and 0.089 ± 0.086, corresponding to each of the values of $s_i = 0.4$, 0.5, and 0.6. The point estimates of b do not differ significantly from 0 at the 0.25 level for any of these values of s_i. More importantly, 95% confidence intervals for b include a substantial range of both positive and negative values. To illustrate with $s_i = 0.5$, the data justify rejecting, at the 5% level of significance, an exponential increase in $s_0(t)$ at a rate greater than $0.095 + 1.96(0.087) = 0.266$ or roughly 27% per year or a decrease at a rate larger in magnitude than 8% per year. Thus, the data are consistent with a broad range of values of b including that assumed in (1), $b = 0$.

One might suspect that no real set of only 13 data points could provide significant evidence for a linear trend in $\log s_0(t)$. On the contrary, in work to be reported elsewhere, we find that at several Chesapeake Bay spawning sites other than the Potomac River, and in the bay as a whole, the correlation of $\log s_0(t)$ with t is significantly negative (C. P. Goodyear, J. E. Cohen, and S. W. Christensen, unpublished data). For such populations, with declining survival of eggs, the present model and methods cannot be used directly.

The autocorrelations of the residuals from the above linear regression at lags 1, 2, and 3 are not significantly different from 0 (the maximum of the t-ratios was 1.9) and decline in magnitude with increasing lag. Thus, there is no significant evidence for correlations in survival over time.

To test whether the variance of $\log s_0(t)$ changes over time, we computed the residuals of $\log s_0(t)$ from the regression line

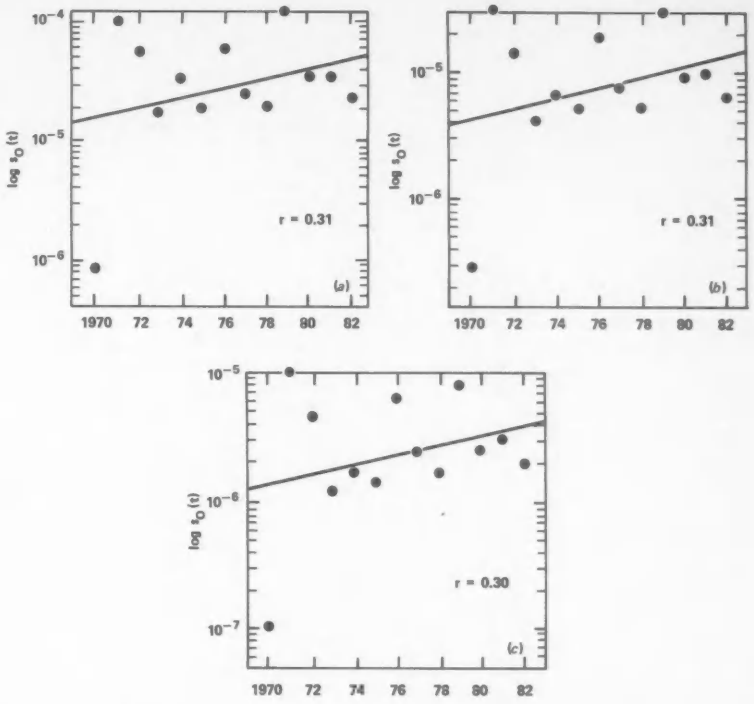

FIG. 1. Survival proportion of eggs to age 1 yr, plotted on logarithmic scale, from 1970 to 1982, at the Potomac River breeding site of the Chesapeake Bay striped bass at (a) $s_i = 0.4$, (b) $s_i = 0.5$, and (c) $s_i = 0.6$. r, linear correlation coefficient between the logarithm of the survival proportion and the year; s_i, assumed annual survival proportion of post-egg females.

$a + bt$, took the absolute values of the residuals, and fitted a regression line of the form $c + dt$ to the absolute values of the residuals. If the variance were independent of time, d would not differ significantly from 0. For all three values of s_i, the estimates of d fall between -0.11 and -0.12, with standard errors between 0.047 and 0.051. The probabilities that d differs from 0 by chance alone, in a two-tailed test, all lie between 0.025 and 0.05. The variance of log $s_0(t)$ may change with t, but the evidence is not strong. The apparently large variance for early t arises from just two data points rather than from many.

The computation of log μ does not depend on whether the variance in survival of eggs is independent of time, but the approximation (29) to the variance of log μ does presume that $\mathrm{Var}(s_0(t))$ is independent of t.

We conclude that, for the Potomac River, the evidence against the assumption that $s_0(t)$ is independently distributed over time with constant mean is statistically weak. Because of the limited number of years of observation, we do not exclude the possibility that the average $s_0(t)$ increases by up to 26% or decreases by up to 8% a year. We also do not exclude the possibility that a lack of independence over time in the true survival probabilities is masked statistically by large, independent sampling variation.

How is the Survival Proportion of Eggs Distributed?

Given that $s_0(t)$ is approximately independently, and approximately identically, distributed over time, what is its distribution? This question arises because we want to test whether the sample mean $\bar{s}_0$ differs significantly from the survival proportion s required for replacement, and it would be convenient to use a test based on statistical theory that presupposes a normal distribution.

We apply two tests of normality implemented in the program library SAS Version 79.5 at Stanford University's computing center: the test of Shapiro and Wilk (1965) (PROC UNIVARIATE) and the Kolmogorov–Smirnov test using Lilliefors' (1967) table of critical points for testing normality when mean and variance are estimated from the sample (PROC KSLTEST). According to Stephens (1974, p. 735), the Shapiro–Wilk statistic provides a more powerful test of normality when the mean and variance must be estimated from the data than the best known statistics based on the

empirical distribution function. This means that if data are non-normally distributed, the Shapiro–Wilk statistic will generally give a "more significant" result (a lower value of the probability P of the null hypothesis of normality) than will the Kolmogorov–Smirnov test. Our results from applying both tests to our data are consistent with Stephens' finding.

For all three values of adult survival s_i, both tests reject the null hypothesis that $s_0(t)$ is normally distributed at probability levels between 0.01 and 0.05. Normal probability plots (not shown here) appear curved. (They should be nearly straight for normally distributed data.)

The model (19) suggests that log $s_0(t)$ should be approximately normally distributed. The Shapiro–Wilk test rejects the normality of log $s_0(t)$ for all three s_i at probability levels less than 0.05. However, the Kolmogorov–Smirnov test rejects normality of log $s_0(t)$ at the 0.01 level when $s_i = 0.4$ and fails to reject normality of log $s_0(t)$ at greater than the 0.10 level when $s_i = 0.5$ and 0.6. Normal probability plots of log $s_0(t)$ (also not shown) appear more nearly straight. We infer that $s_0(t)$ is more nearly lognormally distributed than normally distributed, but the fit to neither distribution is very good.

Is the Mean Survival Proportion of Eggs Different from the Proportion Required for Replacement?

We can compute the proportion s of eggs that would have to survive to age 1 yr for the deterministic population with $L(t)$ given by (2) and $s_0(t) = s$ to be stationary (constant population in the long run). The value of s that gives the Leslie matrix a dominant eigenvalue of 1 may be computed by a formula of Van Winkle et al. (1974) and Vaughan and Saila (1976). The power method may be used to confirm that this value of s gives the Leslie matrix a dominant eigenvalue of 1. With adult survival $s_i = 0.4$, we found $s = 6.93 \times 10^{-5}$; with $s_i = 0.5$, we found $s = 2.11 \times 10^{-5}$; with $s_i = 0.6$, we found $s = 6.82 \times 10^{-6}$.

In the long run, the average population size $E(Y(t))$ will increase or decline if and only if log $\mu > 0$ or log $\mu < 0$, which in turn will hold if and only if $E(s_0(t)) > s$ or $E(s_0(t)) < s$, in the model (1). We now test whether $\bar{s}_0$, the sample mean survival proportion of eggs and the maximum likelihood estimator of $E(s_0(t))$, differs significantly from the survival proportion s required for replacement.

For the cases $s_i = 0.4$, 0.5, and 0.6, the respective sample mean survival proportions 4.48×10^{-5}, 1.24×10^{-5}, and 3.75×10^{-6} are all less than the respective survival proportions s required for replacement, namely, 6.93×10^{-5}, 2.11×10^{-5}, and 6.82×10^{-6}, but the difference is less than one standard deviation, respectively, 3.72×10^{-5}, 1.02×10^{-5}, and 3.22×10^{-6}. Regardless of distributional assumptions, the evidence that $E(s_0(t))$ is less than s appears weak.

Since log $s_0(t)$ is approximately normally distributed (at least for $s_i = 0.5$ and 0.6), it would be tempting to apply the t-test to the sample mean of log $s_0(t)$. However, this would be equivalent to testing whether the geometric, and not the arithmetic, mean of $s_0(t)$ differs from s, which is the wrong question.

In the appendix, an asymptotic likelihood ratio test is derived to test the null hypothesis that a sample of observations comes from a lognormal distribution with fixed mean versus the alternative hypothesis that the observations come from a lognormal distribution with some other mean.

Here, the observations are $s_0(t)$, $t = 1970, \ldots, 1982$, and the fixed mean is the survival proportion s required for replacement, each calculated assuming the same value of the post-egg survival proportion s_i. For all three cases $s_i = 0.4$, 0.5, and 0.6, we find from (A6) that $-2 \log LR$ is smaller than 0.8. The probability of a greater value of $-2 \log LR$ by chance alone, assuming $E(s_0(t)) = s$ and $s_0(t)$ is lognormally distributed, exceeds 0.3. The data do not provide statistically significant evidence that the mean survival of eggs $E(s_0(t))$ differs from s.

The test used is strictly valid only in the limit as the sample size approaches infinity; however, since the value of P is not on the borderline of significance, it seems safe to accept the conclusion of no significant difference between $\bar{s}_0$ and s in this case. The conclusion that the average survival of eggs does not differ significantly from the deterministic level required to sustain a stationary population is robust with respect to the assumed value of the post-egg annual survival proportion s_i.

How might the conclusion of no significant difference between $\bar{s}_0$ and s be affected if the young-of-year data in Table 2 add sampling variability to the biological variability? Substituting (31) into (32) and (32) into (33) shows that $s_0(t)$ is a ratio of random quantities. The numerator is the observation at t. The denominator is a linear combination of observations prior to t. The variance of $s_0(t)$ due to sampling and biological variation will be larger than the variance due to biological variability alone. A small real difference between $E(s_0(t))$ and s may be masked if sampling variability inflates the estimated variance of $s_0(t)$. The conclusion of no statistically significant difference between $\bar{s}_0$ and s must be regarded with substantial caution. Nevertheless, our finding (C. P. Goodyear, J. E. Cohen, and S. W. Christensen, unpublished data) of a statistically significant decline in $s_0(t)$ with increasing time t at other spawning sites in the Chesapeake Bay shows that real biological trends can dominate whatever sampling variability there is in the young-of-year data and gives grounds for modest confidence in the results here.

Growth Rate of Mean Population Size and Its Approximate Variance

If there is no significant evidence that the mean annual survival proportion of eggs differs from that required for the population to be stationary in the long run, then an interval estimate of the growth rate log $\hat{\mu}$ of the average population size should include 0.

By way of illustration in the case $s_i = 0.5$, we find, from the power method, that the dominant eigenvalue of $\bar{L}$ is $\hat{\mu} = 0.927$ and log $\hat{\mu} = -0.076$. Following the procedure under Approximate Standard Deviation of the Estimate of a Growth Rate, and thereby supposing that $\mathrm{Var}(s_0(t))$ is independent of t, we scale v so that its elements sum to 1 and obtain approximately $u_1 = 10570$, $v_0 = 0.99997$, $\mathrm{Var}(\bar{s}_0) = \mathrm{Var}(s_0(t))/(13)^{1/2} = 2.89 \times 10^{-11}$, and finally from (29), $\mathrm{Var}(\log \hat{\mu}) = 3.76 \times 10^{-3}$. The standard deviation of the estimate log $\hat{\mu}$ is 0.061. The estimate log $\hat{\mu} = -0.076$ therefore differs from 0 by little more than one (approximate) standard deviation and has an approximate 95% confidence

interval from −0.198 to +0.046. The absence of strong evidence here that log μ differs from 0 is consistent with the absence of strong evidence that $E(s_0(t))$ differs from s. Because of sampling variability, the evidence is consistent with a substantial rate of decline or relatively small rate of increase in average population size.

When we introduced our numerical estimates of the presumably fixed parameters of the striped bass projection matrix $\boldsymbol{L}$, we raised the possibility that both the post-egg survival proportions and adult fecundity may vary in time. What impact might such variations have on our point and interval estimates of log μ?

Provided the variations in elements of $\boldsymbol{L}$ are independent over time, and provided our numerical estimates are the means of the corresponding random variables, the point estimate of log μ will be completely unaffected by the variation because μ is the spectral radius of the mean matrix $E(\boldsymbol{L}(1))$.

However, the approximate variance of log $\hat{\mu}$ in (29) is computed on the assumption that only $s_0(t)$ varies, neglecting any other source of variation, and is proportional to the variance of $\bar{s}_0$. To the extent that (29) omits additional terms that reflect variation in elements of $\boldsymbol{L}$ other than $s_0(t)$, our approximate variance of log $\hat{\mu}$ is too small. In addition, since the estimate of $s_0(t)$ from (31)−(33) omits any possible variation in s_i and F_i, our estimate of Var($\bar{s}_0$) could also be too small. We conclude that our Var(log $\hat{\mu}$) from (29) will understate the actual variance of log $\hat{\mu}$ if post-egg survival and adult fecundity vary independently over time. A true interval estimate of log $\hat{\mu}$ would, in this case, be wider than that just estimated.

Average Growth Rate of Population Size

To estimate numerically log λ, defined by (5), one procedure is to find the long-run invariant distribution of $\boldsymbol{y}(t)/Y(t)$ and use that to compute log λ as the expectation of $\log[Y(t+1)/Y(t)]$. (Recall that $\boldsymbol{y}(t)$ is the age census, a vector, and $Y(t)$ is the total population size, the sum of the elements of $\boldsymbol{y}(t)$, at time t. Thus, $\boldsymbol{y}(t)/Y(t)$ is the normalized age census, i.e. the probability distribution of individuals according to age.) This procedure is described in Cohen (1977b, 1979b) and illustrated in Cohen (1977b, 1979c) for 2 × 2 matrices. It does not appear to be practical for matrices as large as 15 × 15.

A second procedure would use the mathematical fact that, for any given sample path or simulation, as t gets large, $(1/t)\log Y(t)$ approaches the right side of (5) with probability 1. We start a simulation from some arbitrary initial age census $\boldsymbol{y}(0)$; we use an initial age census with one individual in each age-class. To iterate (1), we specify $\boldsymbol{L}(t)$ by setting $s_i = 0.5$, $i = 1, \ldots, 14$, and by choosing each value of $s_0(t)$ from the corresponding column of Table 2 independently over time with probability 1/13. For t equal to integer multiples of 20, we print the values of $(1/t)\log Y(t)$. Unfortunately, with a single simulation, one has no estimate of the variance of the estimator for, say, 1000 yr, namely $(1/1000)\log Y(1000)$, and running many simulations would be expensive.

In a third procedure, we take as $\boldsymbol{y}(0)$ a scalar multiple of $\boldsymbol{y}(400)$ obtained from a previous simulation. This choice eliminates any transient effects due to a possibly atypical initial age census. We iterate (1) exactly as above and at each step compute

$$(34)\quad r(t) = \log[Y(t)/Y(t-1)],\ t = 1, 2, \ldots, 1000.$$

Then we take as our estimator the sample mean

$$(35)\quad \log\hat{\lambda} = (1/1000)\sum_{t=1}^{1000} r(t)$$

and compute the standard deviation of log $\hat{\lambda}$ in the ordinary way as the standard deviation of the sample mean of $r(t)$. We find log $\hat{\lambda} = -0.086$. The standard deviation of log $\hat{\lambda}$ (not the standard deviation of $r(t)$) is 0.0039. One can prove that, as the duration of the simulation (in this case, 1000) gets arbitrarily large, the sample mean of $r(t)$ approaches log λ with probability 1 and the standard deviation of this sample mean approaches 0. The inequality $\log\hat{\lambda} = -0.086 < \log\hat{\mu} = -0.076$ is consistent with the inequality (12).

Although log $\hat{\lambda}$ is more than three standard deviations below 0, this is no evidence that log $\lambda < 0$, because the estimator (35) takes as given the particular 13 values of $s_0(t)$ on which the simulation is based. Even assuming that our model is correct, if we had happened to observe another 13 yr, we would have found different values of $s_0(t)$ and a different estimate of log $\hat{\lambda}$. An estimate of the variability of log $\hat{\lambda}$ that allows for the variation in $s_0(t)$ could be computed using the jackknife or bootstrap (Efron 1982).

Robustness of Conclusions With Respect to Possible Outlier

According to Fig. 1 and Table 2, the survival proportion of eggs in 1970 is notably lower than the survival proportion $s_0(t)$ for all t after 1970. (It might seem paradoxical that $s_0(1970)$ should be lower than all subsequent values of $s_0(t)$ while the index of young-of-year caught in 1970 exceeds all subsequent indices of young-of-year. However, in (33), the numerator of $s_0(1970)$, namely $y_1(1970)$, is the index of young-of-year caught in the preceding calendar year, 1969, which is only 0.2, less than any subsequent value. The denominator of $s_0(t)$ reflects the size of the spawning stock, which changes slowly over time compared with $y_1(t)$. It is therefore not surprising that $s_0(1970)$ is lower than all subsequent values of $s_0(t)$.)

Here, we investigate whether the previous analyses would have reached different conclusions if this possibly exceptional point for 1970 had been omitted. In linear regressions of log $s_0(t)$ against t with 1970 omitted, the slope coefficient does not differ from 0 at the 0.5 level. For adult survival s_i of 0.4, 0.5, and 0.6, the slope coefficient and its standard error is -0.036 ± 0.056, -0.029 ± 0.058, and -0.029 ± 0.062. Thus, 95% confidence intervals do not exclude both positive and negative slopes. The autocorrelations of the residuals from these linear regressions at lags 1, 2, and 3 are not significantly different from 0, for adult survival $s_i = 0.4$, 0.5, and 0.6. In regressions of the absolute values of the residuals against time, the slope coefficients do not differ significantly from 0 (the values of t are all less than 2). The corresponding values of the slope and its standard error are -0.028 ± 0.031, -0.044 ± 0.029, and -0.056 ± 0.029. Thus, when 1970 is omitted, any suggestion that the variance of the residuals

declines with time is greatly weakened. We conclude that with 1970 omitted, $s_0(t)$ is more nearly independently distributed over time with constant mean and constant variance.

With 1970 omitted, both tests of normality indicate that $s_0(t)$ is not normal ($P < 0.05$ for the Kolmogorov–Smirnov test and $P < 0.02$ for the Shapiro–Wilk test, where P is the probability of the null hypothesis that $s_0(t)$ is normally distributed) but that the normality of log $s_0(t)$ cannot be rejected ($P > 0.15$ for the Kolmogorov–Smirnov test and $P > 0.25$ for the Shapiro–Wilk test). Thus, the lognormal distribution describes acceptably the distribution of $s_0(t)$ when 1970 is omitted, for $s_i = 0.4$, 0.5, and 0.6.

To test whether the mean survival of these lognormal distributions differs from the mean survival proportion s required for replacement, we apply the likelihood ratio test from the appendix as before. For $s_i = 0.4$, 0.5, and 0.6, the values with 1970 omitted of -2 log LR and P are, respectively, 2.46 ($0.1 < P < 0.25$), 3.19 ($0.05 < P < 0.1$), and 3.42 ($0.05 < P < 0.1$). We conclude, as before, that there is no strong evidence, even when 1970 is omitted, that the average survival of eggs differs significantly from the deterministic level required to sustain a stationary population.

We have shown that all our substantive conclusions are robust with respect to the unknown post-egg survival proportion s_i and with respect to the presence of a possible outlier, the data in 1970.

Summary of Data Analysis: Procedure and Results

We summarize the major steps involved in estimating log μ, the long-run growth rate of average population size of an age-structured population with random survival of eggs to age 1 yr. We start with estimates of all elements of the Leslie matrix except the survival of eggs and a time series of the number of 1-yr-olds in the population. The time series must be significantly longer than the maximum reproductive age. We construct a time series of age censuses for the post-egg population, compute the number of eggs produced at each time for which we have a census, and by comparison of the number of eggs produced with the number of 1-yr-olds estimate the proportion of eggs that survive from one year to the next. The average of this survival proportion gives one element in the average Leslie matrix. The logarithm of the dominant eigenvalue of this average Leslie matrix gives log $\hat{\mu}$, when the successive egg survival proportions are independently distributed with constant mean.

When performing this estimation procedure, it is crucial to test its assumptions against the data. Specifically, the assumption that the survival proportions are independently distributed over time with constant mean should be tested by all available means. If the survival proportions are used to make statistical inferences about whether the mean population size is changing the distributional assumptions underlying whatever statistical test is used should be checked and confirmed. Use of the estimation procedure without checking the assumptions that justify it may well mislead.

We also summarize the substantive conclusions we have reached about the striped bass population spawning in the Potomac River. On the basis of estimates of the age-specific fecundity of female fish and an assumed annual survival proportion of post-egg fish equal to 0.5, the fraction of newly spawned eggs that must survive one year to maintain a stationary population is estimated at 2.11×10^{-5}. (As the post-egg annual survival proportion ranges from 0.4 to 0.6, the annual survival proportion of eggs that is necessary to maintain a stationary population ranges from 6.93×10^{-5} to 6.82×10^{-6}.) When the adult female stock is reconstructed from the survival of successive young-of-year cohorts, the input of eggs and hence the annual survival to yearlings can be estimated for each year. For annual post-egg survival proportions between 0.4 and 0.6, the annual proportion of eggs that survive to yearlings, denoted by $s_0(t)$, has a sample mean $\bar{s}_0$ that is less than but does not differ significantly from the value required to maintain a stationary population. Provided that the variance of $s_0(t)$ is not substantially inflated by sampling variability, and provided that the conditions that are assumed in our model were to continue to hold indefinitely, it would be statistically conservative to conclude that the asymptotic annual rate of change log $\hat{\mu}$ in the average size of populations statistically identical to that of the striped bass spawning in the Potomac River lies somewhere in the approximate 95% confidence interval from -0.198 to $+0.046$. The average size may be decreasing by as much as 20% or increasing by up to 5% annually.

Recall from (12) that the almost sure growth rate (log λ) of any single population is always less than or equal to the growth rate of mean population size (log μ). It would be ecologically and statistically conservative practice to continue sampling the young-of-year annually and to attempt to refine the estimates of post-egg survival and of adult fecundity in order to determine whether the suggestion of decline in the average population is happenstance or a genuine problem.

The assumption in our method to which our results are most vulnerable is that adult annual survival proportions and age-specific fecundities have been constant since 1954. Systematic changes in these parameters with time would cause bias in our estimates of $s_0(t)$ that would increase with time.

For example, a trend of decreasing survival or decreasing age-specific fecundities of successive cohorts of adults with time would cause a systematic temporal bias in the ratio of the model-generated egg production to the real egg production. In this situation, the model egg production would overstate the true egg production and thereby underestimate egg survival by a greater fraction each year, which could result in a negative correlation of egg survival with time. Given the apparent increase in angler participation in the region over the period, a decline in the bass population, if found, could have been due entirely to increasing fishing mortality.

Systematic increases in survival or age-specific fecundities of successive cohorts would have the opposite effect. The true slopes of the regressions of egg survival against time would be decreased relative to those calculated if constant vital rates are assumed. The values of $s_0(t)$ would be increasingly overestimated with time by our method. However, the true mean value of egg survival required for replacement would decrease, and its departure from the value s calculated assuming constant adult survival and fecundity would occur at a faster rate than the bias in the estimates of $s_0(t)$ would increase with time. A decline in fishing mortality in response to declining stock would introduce this type of error in our

calculations. If some decline in fishing mortality and some increase in age-specific fecundity have occurred, actual egg survival has increased more slowly or has declined more than our analysis indicates, but the mean actual egg survival would still not be significantly below replacement.

Use of These Methods in Environmental Impact Assessment

Mathematical models of population dynamics are central in attempts to evaluate the environmental impact of existing and proposed power plants (Christensen et al. 1976). Models serve to project the state of a population under baseline conditions, prior to plant operation, and under operating conditions. The difference between the baseline and operational projections describes the environmental impact of plants.

The mathematical models described here originated in a protracted regulatory struggle concerning the Hudson River (Christensen et al. 1981). Over a 17-yr period ending in 1980, Consolidated Edison, an electric utility supplying power to the New York City region, and other utilities, engaged in legal battle with the U.S. Environmental Protection Agency (Region II), the U.S. Nuclear Regulatory Commission, the Federal Energy Regulatory Commission, and a number of state agencies and citizens' groups over the effects of existing and proposed power plants on the Hudson River's fish populations, including the striped bass population.

Scientific consultants for the utilities proposed to evaluate the effects of plant operations by fitting a classical deterministic fish population model to time series of data on catch per unit effort. In the course of analyzing this aspect of the utilities' case, scientific consultants for the U.S. Environmental Protection Agency (Christensen et al. 1982) needed to use stochastic age-structured models. The methods described here offer analytically derived techniques, based on testable assumptions, for computing the long-run growth rate of the average population size when the age-specific vital rates are subject to random variations that are independently distributed over time with constant mean. This growth rate of average population size provides an upper bound on the average long-run growth rate of any single population subject to such random variations in age-specific vital rates.

We have applied these techniques to a time series of young-of-year catches per unit effort from the striped bass population spawning in the Potomac River, in conjunction with very approximate estimates of post-egg annual survival proportions and age-specific egg-laying rates. Although there is no statistically significant evidence that average population would, in the long run, be nonstationary, the data are so limited and variable that the average population may in fact be increasing or decreasing. The possibility of decline warrants continued surveillance of that fish population along with efforts to provide a firmer basis for the vital rates whose values now are largely surmised.

The methods described here, and the more general results referred to in the introduction, provide a framework for modeling stochastic variation of vital rates in real age-structured populations.

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Appendix

Likelihood ratio test for the mean of a lognormal random variable (derived by Lynn Gale, Center for Advanced Study in the Behavioral Sciences, 202 Junipero Serra Blvd., Stanford, CA 94305, USA).

We shall derive a test of the null hypothesis that n observed numbers $x_1, x_2, \ldots, x_n$ are a random sample from the lognormally distributed random variable X with given mean $E(X) = M$, versus the alternative hypothesis that the observed numbers are a random sample from a lognormally distributed random variable X with some unknown mean other than M. Under both the null and the alternative hypotheses, the variance of X is assumed to be unknown.

Let $Z = \log X$ and $z_i = \log x_i$, $i = 1, 2, \ldots, n$. Under the alternative hypothesis, if Z is normally distributed with mean μ and variance σ^2, the maximum likelihood estimators $\hat{\mu}$ and $\hat{\sigma}^2$ are, respectively,

$$\text{(A1)} \quad \hat{\mu} = \bar{z} \equiv \sum_{i=1}^{n} z_i/n$$

$$\hat{\sigma}^2 = \sum_{i=1}^{n} (z_i - \bar{z})^2/n.$$

It is important to observe the denominator n (not $n - 1$) in $\hat{\sigma}^2$. Under the null hypothesis,

$$\text{(A2)} \quad M = \exp(\mu + \sigma^2/2) \quad \text{or} \quad \mu = \log M - \sigma^2/2$$

and we seek the maximum likelihood estimator $(\sigma^*)^2$ of σ^2 subject to this constraint (A2). The likelihood function for the observations is

$$\text{(A3)}\quad L = \prod_{i=1}^{n} ([2\pi]^{1/2}\, x_i\sigma)^{-1} \exp\left(-[z_i-\mu]^2/[2\sigma^2]\right).$$

The maximum likelihood estimator $(\sigma^*)^2$ is obtained by setting $0 = \partial \log L/\partial\sigma$. After some simplification we find

$$\text{(A4)}\quad (\sigma^*)^2 = 2[1+\hat{\sigma}^2 + (\bar{z}-\log M)^2]^{1/2} - 2.$$

The likelihood ratio statistic LR is the quotient of the likelihood function of the sample given the constraint (A2) and using the estimator (A4) to the likelihood function of the sample under the alternative hypothesis using the estimators (A1):

$$\text{(A5)}\quad LR = \left[\prod_{i=1}^{n} ([2\pi]^{1/2}\, x_i\sigma^*)^{-1} \exp\left(-[\log (x_i/M) + (\sigma^*)^2/2]^2/[2(\sigma^*)^2]\right)\right]$$
$$\div \left[\prod_{i=1}^{n} ([2\pi]^{1/2}\, x_i\hat{\sigma})^{-1} \exp\left(-[z_i-\hat{\mu}]^2/[2\hat{\sigma}^2]\right)\right]$$
$$= (\hat{\sigma}/\sigma^*)^n \prod_{i=1}^{n} \exp\left(-[\log (x_i/M) + (\sigma^*)^2/2]^2/[2(\sigma^*)^2] + [z_i-\hat{\mu}]^2/[2\hat{\sigma}^2]\right).$$

Then, the log likelihood ratio statistic, defined to be $-2 \log LR$, will be large if it is much less probable that the sample is lognormally distributed with mean M than if the sample is lognormally distributed with some other mean. After some simplification, we find

$$\text{(A6)}\quad -2 \log LR = -2n(\log \hat{\sigma} - \log \hat{\sigma}^* - [\log M]^2/[2(\sigma^*)^2] + \bar{z} \log M/(\sigma^*)^2 + [\log M - \bar{z} + 1]/2 - (\sigma^*)^2/8 - [\hat{\sigma}^2 + \bar{z}^2]/[2(\sigma^*)^2]).$$

Asymptotically (for large sample sizes), $-2 \log LR$ has the distribution of χ^2 with one degree of freedom, so P values may be obtained from a table of the chi-squared distribution.

NOTES

Behavior and Distribution of Larval and Early Juvenile *Homarus americanus*

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COBB, J. S., T. GULBRANSEN, B. F. PHILLIPS, D. WANG, AND M. SYSLO. 1983. Behavior and distribution of larval and early juvenile *Homarus americanus*. Can. J. Fish. Aquat. Sci. 40: 2184–2188.

Field behavioral observations and plankton tows show that American lobster (*Homarus americanus*) larvae are concentrated in downwellings characteristic of shallow sea fronts. Stage IV lobsters swimming at the surface avoided floating weeds and sticks. Seventy percent of laboratory-reared fourth stage lobsters swam near the surface for more than a minute when released but only 25% of fifth stage lobsters did so. When early fourth stage lobsters encountered bottom, they did not remain long in one place, but late fourth and early fifth stage lobsters began to burrow. Late fourth and early fifth stage lobsters were less likely to return to the surface when they reached the bottom. If the bottom was unsuitable (featureless sand), the lobsters resumed swimming. These behaviors appear to be an appropriate mechanism for substrate selection during settlement.

COBB, J. S., T. GULBRANSEN, B. F. PHILLIPS, D. WANG, AND M. SYSLO. 1983. Behavior and distribution of larval and early juvenile *Homarus americanus*. Can. J. Fish. Aquat. Sci. 40: 2184–2188.

Des observations du comportement sur le terrain et des prises de plancton indiquent que les larves de homard d'Amérique (*Homarus americanus*) sont concentrées dans les régions de plongée des eaux caractéristiques des eaux peu profondes de la mer. Les homards au stade IV nageant à la surface évitent les herbes et bâtons flottants. Soixante-dix pour cent des homards élevés en laboratoire jusqu'au stade IV nagent près de la surface pendant plus d'une minute après leur mise en liberté, tandis que seulement 25 % des sujets au stade V se comportent de la même manière. Au début de leur quatrième stade, les homards qui touchent le fond ne séjournent pas longtemps au même endroit, alors qu'à la fin du quatrième et au début du cinquième stade, ils commencent à se terrer. Ils sont moins susceptibles de retourner en surface une fois qu'ils ont atteint le fond. Si ce dernier ne convient pas (sable dépourvu de relief), les homards reprennent leur nage. Ce comportement semble être un mécanisme approprié au choix du substrat au moment de la descente au fond.

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THE distribution and abundance of the larval stages of the American lobster, *Homarus americanus*, have been extensively studied over the past 50 yr (for a review, see Fogarty 1983). The larvae are pelagic and pass through four instars whose durations are temperature dependent. Settling to the bottom occurs midway through the fourth instar. A number of researchers have investigated the influence of environmental parameters on larval behavior (Phillips and Sastry 1980), but the only field observations are those by Ennis (1975) who released laboratory-reared *H. americanus* larvae at different depths in the sea and observed their swimming behavior. The larvae probably are distributed patchily (Stasko 1980; Fair 1980; Harding et al. 1982), which may make appropriate sampling for abundance estimates difficult. The timing of the change from a pelagic to a benthic existence is variable

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(Herrick 1911) and laboratory observations show that the presence or absence of appropriate substrate strongly influences the point during the fourth stage when the lobster will settle and begin to burrow (Cobb 1968; Botero and Atema 1982). To date, all studies of naturally occurring *H. americanus* larvae have been made using plankton nets, and most studies of the behavior during settling have been made in the laboratory. If we are to have a better understanding of the nature of the recruitment, or settling, process behavioral observations must be made in the field.

We report field observations on naturally occurring pelagic lobster larvae and on the behavior of laboratory-reared fourth and fifth stage lobsters released in shallow water where both surface and bottom environments are easily reached. Our interest was to elucidate possible mechanisms that create a patchy distribution of the pelagic larval stages and to gather information on their substrate selection behavior during recruitment to the bottom dwelling stage.

Methods — Studies of the small-scale distribution of lobster larvae were made in Buzzards Bay, Massachusetts, near the entrance to New Bedford Harbor, July 1–21, 1982. At first we sampled floating weed with dipnets and a 0.5-m^2 lift sampler, but this did not prove successful. After visual sightings of lobster larvae in a "foam line" indicative of a downwelling region associated with a headland front (sensu Pringree et al. 1978), we towed a 0.5-m-diameter plankton net with 0.6-mm mesh at the surface. Tows were made in the center of a foam line, 2–5 m outside it, and at haphazardly chosen locations well away from foam lines or weed patches. All tows were made at 3–4 km/h and most lasted 10 min. Samples were preserved in formalin and returned to the laboratory for sorting and enumeration.

Behavior of lobster larvae was observed by divers using mask and snorkel. On three occasions two of us (DW and TG) made observations of naturally occurring larvae in downwellings. The remainder of the behavioral descriptions are from stage IV and V lobsters reared through the third stage at the Massachusetts State Lobster Hatchery and held for several days in individual containers at the University of Rhode Island. We released and observed lobsters that had been in the fourth and fifth stages for known lengths of time. The lobsters were classified as early (1–5 d since molting) or late (6–9 d since molting) fourth stage, or early fifth stage. The early–late dichotomy was based on the approximate 10-d duration of stage IV at the ambient water temperatures of 19–21°C. All the reported observations were made in shallow (10 cm to 2 m) water in Narragansett Bay, Rhode Island, or in Buzzards Bay. Lobsters were followed by a diver until they were lost to sight, or until they had remained in shelter on the bottom for at least 5 min.

Results — Distribution — Stage III and IV lobsters were sighted from the boat and by divers in or near areas where concentrations of foam were seen at the surface. The concentrations of foam appeared to be caused by patterns of tidal current mixing. The foam usually occurred in lines 1–3 m wide and 200–250 m or more long. The lines were often oriented parallel to the direction of the tidal current flow. The orientation of the foam line had no apparent orientation to wind direction. On two occasions we noted that when the wind strength increased, the surface foam disappeared. Associated with the foam at the surface were bits of flotsam including sticks, insects, and small pieces of weed. Large concentrations of floating weed were not a distinguishing feature of these foam lines. Divers swimming across one foam line below the surface reported temperature differences of as much as 2°C (measured with a hand-held mercury bulb thermometer) from one side to the other. The colder water was clearer, while the warmer water was clouded with particulate matter. High concentrations of crab megalopae were observed in the clear, cold water side beneath the foam line to a depth of 1–2 m. These concentrations were not seen on the warm side.

On three occasions we were able to make observations of naturally occurring lobster larvae discovered in a front. Stage III and IV lobsters were easily visible from the surface at a distance of 1–2 m and nearly as easily seen underwater. Third and fourth stage lobsters were seen continually by two divers in the clear, colder water at concentrations of up to four individuals within a field of vision (2–3 m). Lobsters were not seen in the warm turbid water. We did not note sightings of first and second stage larvae although our plankton tows indicated they were present. The larvae swam steadily and strongly anteriorally; the tail flip response was seen only once in response to the diver. Many of the larvae swam right at or within 1 cm of the surface; others were as deep as 1 m. When they encountered foam, they circled it, picking at it with their claws. Stage IV larvae were also seen picking at small objects in the surface film. Fourth stage lobsters were seen carrying crab megalopae, and on two occasions winged insects.

On four dates during July 10–21, 1982, plankton tows were made inside, within 5 m of either side, or well away from obvious foam lines. Because of the serendipitous nature of the sampling program and the small number of tows, it is difficult to justify tests of significance on the data set. We report the data here in summary, but not reduced form. On July 10, tows 10 min long were taken inside and then on either side of two foam lines, yielding the following numbers of lobster larvae (stages I–IV combined):

Inside foam line	Within 5 m
8	1, 0
4	2, 7

On the same day tows were taken in two other foam lines. One, noted as "dense foam," yielded 33 larvae; the other, noted as "very loose," yielded none. A single tow taken away from any foam line yielded two lobster larvae. Eight tows on two subsequent days (July 13 and 16) gave the following results (on some occasions the foam lines were not long enough to sustain a 10-min tow so 5-min tows were made; to standardize the results of those tows the yield was multiplied by 2):

Inside foam line	Within 5 min	Away
0, 8	5, 16	0, 3, 4, 6

On July 21, four additional tows were made; only two larvae were captured and the data are not reported here. Four

TABLE 1. Numbers of early stage IV (1–5 d after molt), late stage IV (6–9 d after molt), and early stage V (1–3 d after molt) lobsters observed and total observation time in each category after release from surface or bottom.

	Early stage IV		Late stage IV		Early stage V, surface
	Surface	Bottom	Surface	Bottom	
N	18	3	17	2	8
Total time (min)	116	25	138	9	64

National Marine Fisheries Service neuston tows (data courtesy of M. J. Fogarty, NMFS, Woods Hole, MA) made at the same location showed a dramatic reduction in lobster larvae abundance from the 1982 peak on July 9 of 443 larvae per 30-min tow to 13 per tow on July 23.

Larval behavior — Sixty-nine larvae were released and observed. Of these, 21 were lost to sight within 1 min and were not included in the analysis, 43 were released at the surface, and 5 were released at the bottom. The number of animals and the total amount of observation time in each category are shown in Table 1

Thirteen of the 18 early stage IV lobsters (1–5 d after molt) released at the surface swam away from the release site near the surface. There were two principal swimming modes: one with the claws apart, most often seen when the animal was close to the surface, and the other with the claws held together and stretched forward when the animal was more than 2 cm below the surface. The claws were also held apart when the animal was investigating or picking at the surface in what appeared to be feeding motions. The claws-together mode was used in fast, directional swimming.

When early stage IV lobsters swimming just below the surface encountered a patch of foam, they circled it, making picking motions with the claws. However, when they encountered floating weed (*Ulva, Spartina, Fucus*) or sticks, they immediately stopped, backed away slightly, then swam around the material, continuing in the same direction of travel as before the encounter. We attempted to control observer position by occasionally taking positions to the side of and ahead of the lobster, as well as behind it. We saw no change in swimming direction correlated with observer position.

Ten of the early stage IV lobsters reached the bottom during the observations. All of these swam and walked rapidly over the bottom in apparently random directions, stopping to investigate rocks and crevices, typically for 30 s or less. All four animals that were counted as sheltering (Table 2) were lost to sight immediately upon entering a crevice, and we do not know whether the lobster stayed there or was just investigating briefly. None were seen burrowing. On five occasions (50%) early stage IV larvae that were released at the surface and swam to the bottom rose again to the surface.

Three early stage IV lobsters were released over cobble–sand bottom. All three swam to the surface after 30 s to 2 min on the bottom. Two of the three made repeated dives to the bottom. All three were lost to sight while swimming at the surface. None showed burrowing behavior, and only one entered a crevice briefly.

Eleven of the 17 late stage IV lobsters (6–9 d after molt) swam near the surface for more than a minute, 5 of the 11 for between 10 and 20 min. Two swam 40–50 m before being lost to sight.

Swimming behavior of the late stage IV lobsters was similar to early fourths. Both swimming modes were seen, and lobsters swimming at the surface made similar investigatory circlings. However, of the 11 lobsters that swam for some time near the surface, only 3 were seen right at the surface, the rest being 2–30 cm below. Those swimming below the surface occasionally rose to the surface and investigated patches of foam. The proportion of late stage IV lobsters that swam to the bottom was almost equal to that of early fourths (Table 2). After reaching the bottom, only two returned to the surface. The proportion of lobsters that never left the surface was similar between early and late stage IV lobsters.

The behavior of the late stage IV lobsters at the bottom was considerably different from the early stage IV lobsters (Table 2). All nine of those that went to the bottom took shelter under a rock, pebble, or shell and four were seen to perform units of burrowing behavior such as bulldoze, pleopod fan, and backwards dig (see Cobb 1971). The remainder were lost to sight as soon as they entered shelter. We were able to follow six animals for several minutes after they reached the bottom. All remained within a small area (radius of 10 cm), occasionally moving from one crevice to another, but not moving in any particular direction. Two spent 3–5 min investigating and walking rapidly over the bottom before entering a crevice and remaining there. Three of the late stage IV lobsters encountered a hermit crab (*Pagurus longicarpus*) while in or near shelter. On two occasions the hermit crab retreated; on the third, the lobster retreated and went to another crevice.

The two late stage IV lobsters that swam to the bottom and then rose again to the surface had been swimming at the surface for 7–8 min before descending. Both encountered a featureless sand–mud substrate when they reached the bottom. After returning to the surface, one swam for 3 min, descended, found a rock, and burrowed; the second swam for 6 min before we recaptured it.

Two late stage IV lobsters were released at the bottom and observed for more than 1 min. A third was recaptured after surface release and released at the bottom. None swam to the surface, two sheltered and began burrowing after exploring for 1–2 min, and the third was exploring on the bottom when lost to sight after 2 min.

Six of the eight stage V lobsters (1–3 d after molt) released and observed for more than 1 min swam directly to the sand–cobble bottom. Once at the bottom, all found shelter quickly and three were then seen burrowing. Little exploratory behavior was seen. The swimming behavior of the two stage V lobsters that stayed near the surface for more than

TABLE 2. Numbers of stage IV and stage V lobsters in several categories of behavior after release at the surface.

	Early stage IV	Late stage IV	Early stage V
While swimming			
Swam away more than 1 min	13	11	2
To bottom within 1 min	5	6	6
Went to bottom during observation	10	9	7
After reaching bottom, back to surface	5	2	0
Disappeared swimming down	4	3	0
Never left surface	4	5	1
At bottom			
No sheltering, no burrowing	6	0	0
Sheltering, but no burrowing	4	5	4
Sheltering and burrowing	0	4	3

1 min was very similar to that described for late stage IV lobsters. One swam near the surface for 20 min, but when recaptured and released near the bottom immediately took shelter under a rock and began burrowing. The second swam near the surface until it contacted a rock in very shallow water, descended along the rock, sheltered, and started to burrow. Sheltering and burrowing behavior of the early stage V lobsters was not obviously different from stage IV lobsters. At the bottom, one encounter with a hermit crab was seen. After touching the hermit crab, the lobster tail-flipped and swam to the surface, encountered an uprooted, floating *Zostera* plant, and entered the crevices in its roots.

Discussion — Oceanic fronts are important factors in determining distribution of organisms (for a review, see Owen 1981). Frontal zones are regions of convergence where vertical motion occurs. They may be characterized by changes in salinity, temperature, velocity, sea state, and accumulations of particulate material (Bowman and Esias 1978). These regions of surface convergence may be caused by a variety of factors, including tidal current mixing, river discharge, and wind-induced Langmuir circulation. The convergence causes downwelling in which lighter than water particles are concentrated near or on the surface. Often they can be visually located by the presence of foam, scum, or floating weed. Alldredge and Hamner (1980) observed aggregations of zooplankton in the lee of a small point projecting into a tidal current and suggested a hydrodynamic explanation for the accumulation. Our observations and plankton tows showed that lobster larvae accumulated in what appeared to be tidal current induced downwellings. We speculate that they may be swept into the downwelling by lateral advection and maintained there by a combination of positive phototaxis and high concentrations of food such as crab larvae and insects. The fronts are ephemeral, appearing or disappearing with changes in currents or wind. Thus, variability in larval concentrations such as we observed would be expected and presumably is dependent on the age of the frontal system.

Normandeau Associates (1980) reported high concentrations of lobster larvae associated with rafts of *Spartina* grasses. Harding et al. (1982) found a correlation between the presence of weed and the presence of larvae in their neuston samples. They suggested that both the weed and the larvae were concentrated by Langmuir circulation and that the weed may provide cover for the dark-colored larvae. Our work showed, with both plankton tows and visual sightings, that the larvae are found in higher concentrations in downwellings, of which Langmuir circulation is only one type. Swimming stage IV lobsters avoided floating weeds and sticks. Thus, we suspect that the association between weed and stage IV lobsters may be fortuitous. It is apparent that further work needs to be done on the behavioral aspects of association between floating objects and lobster larvae. However, the recommendation of Harding et al. (1982) that sampling should be done across the wind because of Langmuir cell induced concentrations is not sufficient. Sampling should be planned to account for all types of frontal systems insofar as possible.

Stage IV lobsters would pause to investigate and pick with the chelipeds at patches of foam and particulate matter floating at the surface. The surface film of the water is rich in organic material (Wangersky 1976), which is concentrated in the surface foam. In addition to zooplankton, lobster larvae also apparently consume insects according to our anecdotal observations and one by Williams (1906). The lobsters may also be using the surface foam as a nutrient source. Scully (1978) showed that hermit crabs feed upon surface foam in very shallow water.

There is a good deal of laboratory evidence concerning the nature of substrate choice by settling stage lobsters, but only direct field observations can provide conclusive evidence concerning the nature of settling and substrate choice. Ennis (1975) released laboratory-reared stage IV and V lobsters at the surface and at the bottom in 9.1 m of water. All but the early stage IV tended to remain at the bottom when released there, and all but the late stage V remained at the surface when released at the surface. He noted that those remaining at the bottom tended to find shelters. Our observations provide detail that helps in the understanding of how the behavioral choice to stay at the bottom or return to the surface is made. Early stage IV lobsters at the bottom did not burrow and were likely to return to the surface, but late stage IV lobsters on suitable bottom sheltered and burrowed. All lobsters that

encountered a sandy or muddy substrate resumed swimming. These observations are consistent with the hypothesis that settling lobsters actively select suitable substrate. The behavior of stage V lobsters was quite predictable; when released at the surface, six of eight swam to the bottom and, once there, they stayed. This differs from the observations of Ennis (1975) in which eight of nine early stage V lobsters stayed at the surface when released there.

An important component of settling behavior appears to be burrowing activity. None of the early stage IV lobsters we observed in the field exhibited burrowing behavior in the few minutes we were able to follow them after they reached the bottom, although burrowing by stage IV lobsters starting approximately 2 d after molt has been described (Berrill 1974; Botero and Atema 1982). It may be that remaining on the bottom is dependent on the ontogenetic development of burrowing behavior.

Competition for scarce resources exists between lobsters and other decapods (Fogarty 1976; Wang 1982; Richards et al. 1983). Competition started literally within minutes of the time the lobsters reached bottom. Four newly settled lobsters encountered hermit crabs, two retained their shelters, and two retreated. Competition (and predation, of which we observed one instance) probably is an important factor affecting the success of settlement and recruitment and needs further field study.

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A Time Series Approach to Forecasting Angling Activity at a Recreational Site Based on Past Successes

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STEINNES, D. N., AND R. L. RAAB. 1983. A time series approach to forecasting angling activity at a recreational site based on past successes. Can. J. Fish. Aquat. Sci. 40: 2189–2193.

A multiple regression model was developed for estimating or forecasting the impact of past fishing "successes" on the current angling activity or participation at a recreational site. Through this approach we demonstrate the usefulness of time series methodology as a forecasting tool by investigating the lag structure of success and measuring its elasticity. We evaluated empirical results, based on daily time series data for a Lake Superior river; these indicate that anglers respond more to past total success at the site than to past success per unit of effort at the site. Finally, we examined how the model could be extended so as to estimate the economic value of catching a fish and how, in turn, such an extended model might be part of a comprehensive approach for valuing changes in habitat using benefit-cost analysis.

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Un modèle de régression multiple a été mis au point dans le but d'estimer ou de prédire la répercussion de « succès » de pêche passés sur les présentes activités de pêche de sport à un site récréatif. Nous démontrons, avec cette approche, l'utilité de séries chronologiques comme outils de prédiction en étudiant les caractéristiques du décalage du succès et en mesurant son élasticité. Nous avons évalué les résultats empiriques fondés sur une série quotidienne de données recueillies dans une rivière du bassin du lac Supérieur; ces résultats indiquent que les pêcheurs à la ligne réagissent davantage aux succès passés au site qu'aux succès par unité d'effort à ce site. Finalement, nous examinons la façon dont le modèle pourrait être élargi pour estimer la valeur économique de la capture d'un poisson et comment, à son tour, un modèle ainsi élargi peut être partie d'une approche générale de l'évaluation des changements d'habitat à l'aide d'une analyse de coût-bénéfice.

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IN fisheries management, decisions must be made that affect the availability of fish for recreational anglers. Most obvious are decisions concerning the stocking of a habitat with one or more species; but, in addition, indirect decisions affecting water quality, such as banning the discharge of an effluent in a stream, may also affect the availability of fish. These decisions would likely be made on the basis of cost-benefit analysis (Mishan 1976), since recreational angling is a public and/or a free good in most areas. While determination of costs associated with decisions such as stocking are rather straightforward, the estimation of benefits is a more difficult task. It seems reasonable to presume that a decision such as stocking would increase the availability of fish and angling success in the habitat, although the actual relationship involved would have to be estimated empirically and probably over an extended period of time. Given that success can be changed by fisheries management decisions, it is the intent of this paper to investigate whether anglers respond positively to increased success. If they do not, then even though changes in habitat may generate other benefits, such changes that improve angler success will not have any recreational angling benefits. Although the model indicates a definite angler response to success, it is still necessary to extend the model in order to measure the economic value of this angler response and to develop a comprehensive approach for valuing angling benefits deriving from changes in habitat quality using benefit-cost analysis.

While our study only attempts to measure the effect of success on angler activity, other studies have investigated the effect of success on anglers' willingness to pay or the value of success. Stevens (1966) introduced the notion of the quality of the fishing experience as a determinant of angler effort by emphasizing the importance of angler success. He reasoned that improvements in success (e.g. by effective fishery management or habitat improvement) can yield measurable economic benefits. By estimating the increasing responsiveness of angling demand with respect to success when comparing daily data with weekly data, Stevens (1966, p. 175) surmised that a time lag was involved in the transmission and receipt of information on changes in success elasticity. Although he was the first to measure current elasticity, he used only one measure of success and did not employ lagged variables.

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The method that we employ is called distributed lag and allows for empirical determination of the effect of past values of an independent variable (angler success) on the current value for the dependent variable (angling activity). Using daily time series data we determine the elasticity of angling demand with respect to success (i.e. how responsive anglers are, in terms of the amount of angling they do, to success) and the lag structure (i.e. how fast anglers stop responding to past successes). Econometric models that employ time series methodology are widely used as a forecasting tool but have not been often applied in recreational studies. Such forecasting models are especially useful because, using current information for the lagged variables, it is possible to forecast future activity. This approach has added usefulness as a management tool.

Forecasting model of angling participation — Our model, a multiple regression equation, was estimated using daily time series data. We estimate this model for predicting or forecasting angler activity or usage at a site based on past "successes" at the site and with time series data for the Lester River along the north shore of Lake Superior. This river is located within the city limits of Duluth, Minnesota, and much of the activity consists of local anglers who fish from shore. These anglers generally do not camp or stay overnight near the river but, rather, they fish on weekends or during the week in the morning or evening. The proximity to Duluth allows many local anglers to fish the river with little preparation and makes them able to react or respond to daily changes in conditions (e.g. weather and success). We believed that this type of fishery afforded us the necessary data to test the type of forecasting model to be presented.

This forecasting model is potentially useful to fishery managers as a tool for forecasting the number of anglers, the dependent variable, at a recreational site on some future day given or assuming values for the independent variables in the model. For example, being able to forecast the number of anglers on a daily basis may facilitate assignments of enforcement personnel or suggest the need for limiting access, perhaps by charging an entry fee on certain overcrowded days (e.g. weekends). Aside from the model's general usefulness as a forecasting tool, the specific interest will be the "success" variables that will demonstrate that the anglers do respond very quickly to success at a recreational site (i.e. the current level of angling activity at a site is determined by the success that occurred at the site in the recent past).

The forecasting model in general terms is as follows: angling activity$_t$ = f(angling success$_{t-i}$, trend, seasonal, weather conditions). For multiple regression estimation the following two general stochastic models, which vary as to how success is defined, were used:

$$(1)\quad \text{ANGLERS}_t = f(\text{TOTALFISH}_{t-i}, \text{DATE}_t, \text{WEEKEND}_t, \text{MEANTEMP}_t, \text{PRECIP}_t, e_{1t})$$

$$(2)\quad \text{ANGLERS}_t = f(\text{CATCHRATE}_{t-i}, \text{DATE}_t, \text{WEEKEND}_t, \text{MEANTEMP}_t, \text{PRECIP}_t, e_{2t})$$

where the following variables are defined. Angling activity variable: ANGLERS$_t$ = number of anglers fishing on day t; angling success variables: TOTALFISH$_{t-i}$ = total number of fish caught on day $t - i$ ($i \geq 0$) and CATCHRATE$_{t-i}$ = fish caught per angler hour on day $t - i$ ($i \geq 0$); trend variable: DATE$_t$; seasonal variables: SAT$_t$ = 1 if Saturday and = 0 otherwise, SUN$_t$ = 1 if Sunday and = 0 otherwise, and WEEKEND$_t$ = 1 if Saturday or Sunday and = 0 otherwise; weather condition variables: MEANTEMP$_t$ = mean temperature on day t and PRECIP$_t$ = precipitation on day t.

Since the model is estimated using daily time series data for a single location (Lester River in Duluth), the variables are defined with a subscript t. The variables TOTALFISH$_{t-i}$ and CATCHRATE$_{t-i}$, which are alternative measures of success at the site on day $t - i$, are introduced in current and in lagged form (i.e. TOTALFISH$_{t-1}$ would be total fish caught on the previous day and TOTALFISH$_{t-2}$ is the same variable for 2 d previous). These lagged variables will be used to determine if the current (t) quantity of angling activity is responsive (positively) to current (t) and/or previous successes at the site ($t - 1$, $t - 2$). The other variables are introduced to account for trend (DATE), seasonal (SAT, SUN, WEEKEND), and weather conditions (MEANTEMP, PRECIP), while e_{1t} and e_{2t} are random disturbances satisfying the standard statistical assumptions. Discussion of the signs of these variables will be given with presentation of the estimation results.

Primary interest is focused on the success variables, and it will be found that the level of angling activity (ANGLERS) is more responsive to lagged TOTALFISH than lagged CATCHRATE. Further, it will be discovered that success on the previous day is about 10 times more important than success 2 d prior, which suggests that "the word" spreads fast among anglers and that it must be current.

Whereas the relationships in equations 1 and 2 to be estimated will determine the existence and extent of an angler response to success at a recreational site, the economic value of this response could only be hypothesized from these results. One possibility for valuing success might be to estimate a demand relationship for angling with success incorporated as an exogenous or shift variable. For example, the Clawson "travel-cost" method procedure (Clawson and Knetsch 1975) for recreational demand estimation might be used, but this would require that information be available, which it is not, on the home location of the anglers on each day. If such data were available, it might be possible to determine if the angler response to success at the site is related to distance or not. Based on the results on a localized fishery, we suggest that local anglers are responsive in the short run (i.e. daily changes) to changes in success. It may be that long-run responsiveness (i.e. to changes in success that are more permanent) to success is less localized. Using the travel-cost method the latter type of response (i.e. increased nonlocal angler activity) would be valued higher than local response. These issues are not resolved here, but we suggest that there is a definite short-run positive response in angler activity to success regardless of how the economic value of this response is to be measured.

Estimation of the forecasting model — Our data base was obtained from personnel at the French River Hatchery, Minnesota Department of Natural Resources, and was collected as part of their Lake Superior creel census program. The Lester River in Duluth was surveyed in 1977 during the

TABLE 1. Angler response to total fish caught (TOTALFISH) (t values in parentheses).

Independent variable	Equation				
	1.1	1.2	1.3	1.4	1.5
DATE	−0.17	−0.19	−0.19	−0.17	−0.18
	(−1.68)	(−2.21)	(−2.25)	(−1.56)	(−1.91)
TOTALFISH	0.44				
	(0.87)				
TOTALFISH_{t-1}		1.42	1.42	1.38	1.34
		(3.27)	(3.33)	(3.08)	(2.70)
TOTALFISH_{t-2}					0.11
					(0.20)
SAT	7.21	6.53			
	(1.46)	(1.55)			
SUN	7.70	6.88			
	(1.95)	(2.03)			
WEEKEND			6.75	6.77	6.58
			(2.38)	(2.31)	(2.14)
PRECIP				−3.41	−3.60
				(−0.51)	(−0.54)
MEANTEMP				−0.02	−0.01
				(−0.11)	(−0.09)
Constant	43.74	43.14	43.10	42.22	41.71
R^2	0.24	0.44	0.44	0.45	0.45

spring (May−June) steelhead (*Salmo gairdneri*) run on a regular basis to collect a systematic sample of 33 d during the 2 mo. Thus, the model is estimated with only 33 observations and two lagged variables.

We estimated a simple additive (or linear) form using ordinary least squares multiple regression analysis. Whereas other forms have certain advantages (e.g. a multiplicative form provides direct estimates of elasticities), the simplicity of the linear form seemed more appropriate as a management forecasting tool. (While only linear results or forms of equations 1 and 2 are presented, it should be noted that nonlinear functional forms offer certain advantages. For example, introducing (polynomial) nonlinearity in the independent variables would allow for decreasing marginal angler responses to changes in success. It would be convenient for finding elasticity to introduce nonlinearity in the parameters through the use of a multiplicative model ($Y = X_1^a X_2^b$), which can be estimated in log-linear form ($\log Y = a \log X_1 + b \log X_2$) using ordinary least squares; the regression coefficients (a and b) are direct estimates of elasticity. Furthermore, such nonlinear models will give out higher R^2 statistics. For the sake of expositional simplicity, we have chosen to present only linear forms of the equations, since nonlinear estimation led to similar conclusions.)

In examining the results, our focus is on overall explanatory power, which is measured by R^2, and the significance of individual explanatory or independent variables, as measured by t values. Given the time series nature of the data, autocorrelation can present a problem. Therefore, the Durbin−Watson test for the presence of autocorrelation was conducted but proved to be in the indecisive range in most equations; thus, alternative estimation procedures were not pursued.

Tables 1 and 2 provide comparable results for equations 1 and 2 to determine which of the success variables, TOTALFISH or CATCHRATE, is a more important determinant of angling activity at a site. In both tables, or equations, the dependent variable is the amount of angling activity, which is measured as the number of anglers per day, ANGLERS. Various equations, and the other independent variables, are specified in each table and discussed later in this section. We suggest from the R^2 values that TOTALFISH provides a greater explanation of ANGLERS than CATCHRATE. An ex post interpretation of this is that anglers respond (by going fishing at a site) more to the total number of fish caught at a site than to the success per unit of effort, perhaps because they are more likely to hear of or become aware of the former than the latter. It should be kept in mind that these results are based on daily information. This is not to say that an individual's angling activity for a season is determined by the success at the location the previous year but that the angler chooses to fish on a given day (and, hence, the amount of daily aggregate activity at the site, ANGLERS, increases) when there has been success (i.e. high TOTALFISH_{t-1} or CATCHRATE_{t-1}) at the site the day before. Since the previous day's success is also measured in aggregate terms (i.e. based on all activity the previous day), it follows that the model assumes that an angler reacts to other's success (i.e. the information as to others' success at the site is transmitted daily), not to his/her own past success. Also, the short-run (daily) nature of the data and model is not intended to determine the long-run effect (i.e. the possible decrease in success with increased activity) of angler activity on success. To do this would require a simultaneous equation model and long-term (e.g. annual) data collection.

Another major interest in this study was to determine something about the reaction time of anglers to past success at the site. In equations 1.1 and 2.1 (Tables 1 and 2) the current, rather than past, values for success are included, since this was the modeling approach used by Stevens (1966, p. 175) to measure success elasticity in both daily and longer run time series estimates. In our results, current success proves to be insignificant, which is reasonable given the short-run (daily)

TABLE 2. Angler response to fish caught per hour (CATCHRATE) (t values in parentheses).

Independent variables	Equation				
	2.1	2.2	2.3	2.4	2.5
DATE	−0.18	−0.19	−0.19	−0.17	−0.18
	(−1.77)	(−1.94)	(−1.97)	(−1.66)	(−1.63)
CATCHRATE	−16.83				
	(−0.84)				
$CATCHRATE_{t-1}$		33.89	33.37	30.84	29.72
		(1.76)	(1.77)	(1.56)	(1.35)
$CATCHRATE_{t-2}$					2.92
					(0.13)
SAT	7.41	7.09			
	(1.51)	(1.50)			
SUN	8.10	8.37			
	(2.05)	(2.20)			
WEEKEND			7.90	7.88	7.71
			(2.49)	(2.45)	(2.17)
PRECIP				−3.72	−4.08
				(−0.51)	(−0.51)
MEANTEMP				−0.05	−0.02
				(−0.02)	(−0.01)
Constant	47.91	44.68	44.56	42.95	42.90
R^2	0.24	0.30	0.30	0.31	0.31

nature of our data, whereas with annual data, one would expect success and angler activity to be highly correlated. Thus, on a contemporaneous daily basis, success may be determined more by other exogeneous factors (e.g. availability of anadromous fish in heavily fished areas) than by the level of current participation. When lagged, or past, values are used in equations 1.2−1.4 and 2.2−2.4 they prove to be more significant than current values although $TOTALFISH_{t-1}$ is more significant than $CATCHRATE_{t-1}$. This confirms that the angling activity (at time t) is responsive to success at time $t-1$. However, when the success at time $t-2$ is introduced, in equations 1.5 and 2.5, it is positive but only about one-tenth as important (e.g. the coefficient is 0.11 for $TOTALFISH_{t-2}$ but 1.34 for $TOTALFISH_{t-1}$) as the success at time $t-1$. This suggests that in this particular situation, anglers fish, or participate, at time t more so on the basis of the previous period's ($t-1$) success than $t-2$. With further data and greater lags it would be possible to determine more exactly the rate of decline in explanatory power of the lagged variables or the lag structure.

Since it has been found that success at time $t-1$ is most important in determining angling activity at time t, the meaning of the regression coefficient (b) should be explained. In equation 1.2, $TOTALFISH_{t-1}$ has a coefficient of 1.42, which suggests that every fish caught at time $t-1$ will lead to 1.42 more anglers at time t. This direct interpretation of the regression slope coefficient (b) in a linear equation may be supplemented by finding the success elasticity, or responsiveness, of angler activity to previous success (fish caught). Elasticity (E) is often used because it is unit-free and indicates the percent change (%Δ) in the dependent variable, Y, that will result from a 1% change in an independent variable, X:

$$(3)\quad E = (\%\Delta Y)/(\%\Delta X) = (\Delta Y/\Delta X)(X/Y).$$

The elasticity equation 3 can be determined from the estimated linear model by inserting the estimated regression coefficient for $(\Delta Y/\Delta X)$ and substituting the mean values ($\bar{X}$ and $\bar{Y}$) for X and Y. For equation 1.2 the substitution is

$$(4)\quad E = (\Delta Y/\Delta X)(X/Y) = b(\bar{X}/\bar{Y})$$
$$= 1.42(3.72/26.7) = 0.2.$$

The interpretation of this value is that a 1% increase in success (fish caught) at time $t-1$ will lead to a 0.2% increase in anglers at time t. This figure is relatively low and suggests that angler response is rather success inelastic.

While the primary purpose of the model is to examine the responsiveness in angler activity to success, other variables (trend, seasonal, and weather condition) have also been used and appear in Tables 1 and 2. The variable DATE is a simple trend variable, and its negative coefficient indicates that angling was declining through the sample period, which may suggest that angler activity is high at the beginning of the fishing season and then falls over time. Originally, SAT and SUN were introduced as separate dummy variables to account for the increased angling activity on weekends. When it was discovered that these variables had similar coefficients (see equations 1.1, 1.2, 2.1, and 2.2) a single dummy variable, WEEKEND, was used in the later results (see equations 1.3−1.5 and 2.3−2.5). As expected, the coefficient for this seasonal factor is positive and significant, indicating increased angling at the site on weekends. If more data were available for a longer period of time, it would be reasonable to take account of holidays and also test for a nonlinear trend.

The variables intended to account for the effects of weather conditions on angling activity met with less success. While PRECIP has the expected sign (i.e. more rain, less angling), it proved insignificant. MEANTEMP was insignificant and had the apparent wrong sign although the poor results for this variable may be the result of limited variation in daily mean temperature over the 2 mo. Data for a whole year would have

greater variation in temperature, which might yield the correct sign and make this variable more significant. The results suggest that anglers are little influenced by weather conditions, or at least much less affected than they are by success the previous period.

Each of the equations in Tables 1 and 2, with the exception of equations 1.1 and 2.1, which have current success as independent variables, could be used for forecasting future angler activity. If the climatic conditions were forecast for a future day, the other independent variables would be known so forecasts could be made into the future. As with any forecasting tool, however, the ability of the model to predict becomes more tenuous when forecasts are made further into the future. Also, the rather low R^2 in Tables 1 and 2 makes forecasting difficult. A better R^2 might be achieved through more data collection (i.e. more days and more explanatory variables) and more complicated model specification (e.g. nonlinear or interaction terms).

Toward the valuation of success — The eventual use of the model may be to provide an estimate of the economic value or benefit of angling success, but at present it does provide a potentially useful forecasting tool for fishery managers in its present form. The model also confirms the importance of a time series approach, since we have found that there is a definite positive response in angling activity to increases in past success at a site. One possible interpretation of this might be that the demand (for angling at the site) relation shifts up, or that there is an increase in consumer surplus, but measuring the exact economic value of this increase would have to be based on demand estimation, perhaps using the travel-cost type models that have already come to include various determinants of angler fishing time, such as family income, distance from the fishery, and transfer costs (Knetsch 1963; Brown et al. 1964). Other studies indirectly have attempted to estimate the value of success based on surveys of anglers in which they are asked to state what dollar value they attach to catching a fish. This methodology (Brown et al. 1978) requires that a survey respondent consider a hypothetical situation, and, consequently, it is similar to past approaches used in estimating the economic value of a recreational day. In the valuation of a recreational day, the Clawson travel-cost method has also been used because it is based on direct observation of individuals' actual participation in a recreational activity and the indirect measurement of their willingness to pay based on the distance (or time) between their home and the recreational site. Our approach for measuring the effect of past success on current angler activity at a site might, if data were available, be used in conjunction with the travel-cost approach.

A simple approach to valuing success, which would not involve the travel-cost method, would be to use the coefficient (1.42) for TOTALFISH_{t-1} in equation 1.3 (i.e. each fish caught at time $t - 1$ will lead to 1.42 additional anglers at time t) and determine the economic value of this increase in anglers using existing estimates (e.g. Talhelm 1978; Talhelm et al. 1979) of the economic value of an angler day. Even though quite naive this approach would at least provide a way of determining the marginal value of success in terms of a recreational activity (angling). One criticism of the travel-cost method as a means of valuing one input (e.g. success or wildlife) is that the one input cannot be isolated from the other inputs, which are combined to produce the recreational experience or activity. The model we provide indicates the marginal amount of activity that will result from increased success and, hence, the activity can be valued as a whole experience by a suitable method and it will still be possible to value success. One potential problem, as explained earlier, is that the response to success may be a "local" one, and this would have to be considered if the Clawson method were to be used. Also, our response, or success elasticity, is a very short-run estimate and it is reasonable to assume that the long-run response would be greater.

While our short-run model provides some insight into the responsiveness of anglers to success, a much more complete analysis would be possible if a long-run demand model were to be estimated; but this would require more extensive data and probably a pooled time series cross-section econometric approach with its inherent statistical problems. In addition, the cost-benefit analysis of such decisions to which the introduction alludes must await the estimation of the relationship between stocking programs or changes in habitat quality and success at a site. This latter biological relationship might eventually be combined with the model provided in order to estimate more completely the economic value of fisheries management programs and decisions.

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Estimation of Disease Incidence in Fish Populations

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WORLUND, D. D., AND G. TAYLOR. 1983. Estimation of disease incidence in fish populations. Can. J. Fish. Aquat. Sci. 40: 2194–2197.

A method is described to estimate the disease incidence in large populations of fish where the material analyzed is composed of a number of pooled individuals. A procedure for calculating a confidence interval estimate is presented, and the bias of the estimate discussed. The methods were developed for estimating disease incidence in hatchery populations of juvenile Pacific salmon (*Oncorhynchus* spp.), but are applicable to any situation in which the sampling plan can be assumed to follow the binomial distribution.

WORLUND, D. D., AND G. TAYLOR. 1983. Estimation of disease incidence in fish populations. Can. J. Fish. Aquat. Sci. 40: 2194–2197.

L'article qui suit contient une description de méthodes permettant d'estimer l'occurrence de maladies dans de grandes populations de poissons pour lesquelles le matériel analysé est constitué par plusieurs individus groupés. On y présente une méthode d'estimation des intervalles de confiance, avec analyse du biais d'estimation. Ces méthodes ont été mises au point en vue d'estimer l'occurrence de maladies chez des populations d'élevage de saumoneaux du Pacifique (*Oncorhynchus* spp.), mais elles sont applicables également à toute situation dans laquelle on peut supposer que le plan de l'échantillonnage suit une distribution binomiale.

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OSSIANDER and Wedemeyer (1973) described a method for determining the sample size needed to detect a given incidence of diseased fish in a population. As an example they presented sample sizes needed for various incidence levels and large population sizes such as a hatchery production of juvenile Pacific salmon (*Oncorhynchus* spp.). Sample sizes were determined so that the probability is no less than 0.95 to have at least one diseased fish in the sample if the disease incidence is at least a specified value. In their example, sample sizes of 60 and 150 individual fish were found adequate for detecting disease incidences as low as 5 and 2%, respectively, when sampling from populations as large as 10^6.

Because of the cost and time associated with disease determinations, individual fish are not usually examined. Instead, material from say five fish are combined and the resulting pool is analyzed; for example, one diseased individual in the sample will cause one of a number of groups of five fish to be disease positive. If all groups are disease negative, the incidence is unlikely (probability of 0.05) to be as great as that originally specified. On the other hand, if some of the groups are disease positive it is of interest to determine the incidence level. Here we develop a method for estimating disease incidence when samples are drawn from a large population and are pooled and analyzed in this manner.

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Methods — Let R be the proportion of a large population of fish that is disease positive. Suppose that n samples, each a group of k fish, are drawn from the population; then $U = 1 - R$ is the probability of obtaining a disease negative individual, U^k is the probability that all fish in a group of k are disease negative, and $P = 1 - U^k$ is the probability that at least one fish in a group is disease positive; i.e., the group of k fish is disease positive.

Let x be the number of groups found to be disease positive in the sample of n groups. Assuming that x is a binomial variate with parameters n and P, the probability of observing exactly x disease positive groups is:

$$(1)\quad PR(x) = \frac{n!}{x!\,(n-x)!}\,(1-U^k)^x\,(U^k)^{n-x}.$$

The maximum likelihood estimate of R is given by:

$$(2)\quad \hat{R} = (1-\hat{U}) = 1 - [1 - (x/n)]^{1/k}$$

To construct a confidence interval estimate for R, where n, x, and k are fixed, consider the following probabilities:

$$(3)\quad PR(m \geq x) = \sum_{m=x}^{n} \frac{n!}{m!\,(n-m)!}\,(1-U^k)^m\,(U^k)^{(n-m)}$$

$$(4)\quad PR(m \leq x) = \sum_{m=0}^{x} \frac{n!}{m!\,(n-m)!}\,(1-U^k)^m\,(U^k)^{(n-m)}.$$

In equation (3) let R_l be the largest value of $R = 1 - U$ for which $PR(m \geq x) \leq a$; and in equation (4) let R_u be the smallest value of R for which $PR(m \leq x) \leq a$. R_l and R_u are then the respective lower and upper limits of a $100(1 - 2a)\%$ confidence interval for R. In practice, a finite number of decimal places for R_l and R_u must be predetermined and equations (3) and (4) solved by trial and error.

Table 1 gives solutions for equations (2), (3), and (4) in situations where samples of 12 and 30 groups of five fish each are obtained. This table is in current use by the Olympia Fish Health Laboratory for sampling hatchery populations of juvenile salmon. The value of a in equations (3) and (4) was set at 0.05 to produce a 90% confidence interval. Copies of computer programs for obtaining the estimates are available from the authors.

In Table 1 the upper limit of the 90% confidence interval is 4.9% for the event of observing zero disease positive groups of five fish from a total of 12 groups. An assertion can be made that for any particular value greater than or equal to 4.9%, the probability of observing zero disease positive groups is less than or equal to 0.05. This agrees well with the disease incidence detected for large populations as determined by Ossiander and Wedemeyer (1973). A similar result holds for the sample of 30 groups of five fish each.

When n (i.e. number of groups) is large and $0 < \hat{R} < 1$ confidence intervals can also be obtained by making use of the asymptotic normality of maximum likelihood estimates (Wetherill 1981). A $100(1-2a)\%$ confidence interval for R is:

$$(5) \quad \hat{R} \pm Z_a[V(\hat{R})]^{1/2}$$

where Z_a is the point of the standard normal distribution and $-1.0/V(\hat{R})$ is the expectation of the second-order derivative of the log of $PR(x)$ with respect to U. The variance of $\hat{R}$, $V(\hat{R})$, can be shown to be given by:

$$(6) \quad V(\hat{R}) \geq (1 - U^k)/nk^2U^{k-2},$$

$$\geq U^2 (U^{-k} - 1)/nk^2$$

and is estimated by substituting $(1 - \hat{R})$ for U. Equality holds if $\hat{R}$ is an unbiased estimate of R.

Using (5) and (6), 90% confidence intervals corresponding to values of $100(\hat{R})$ of 3.6, 7.8, 12.9, 19.7, and 30.1 and for $n = 12$ and $k = 5$ are (0−8), (0−15), (3−23), (7−32), and (12−48), respectively. Similar intervals for $n = 30$ are (0−7), (3−12), (7−19), (12−28), and (19−41), respectively. The upper limits of these intervals, which are generally of most interest in estimating disease incidence, compare favorably with those in Table 1. It appears then that if the number of groups is at least 30, the simpler method given by equations (5) and (6) could safely be utilized.

Except when individuals are examined, the estimate of R given by equation (2) is biased. The expected value of the estimate of R is:

$$E(\hat{R}) = 1 - E(\hat{U}),$$

where

TABLE 1. Estimated percentage disease incidence for 12 and 30 samples of five fish each.

Number of samples	Number samples disease positive	Point estimate	90% confidence limits	
			Lower	Upper
12	0	0.0	—	4.9
	1	1.72	0.08	7.9
	2	3.58	0.62	10.9
	3	5.59	1.48	13.9
	4	7.79	2.59	17.1
	5	10.22	3.92	20.6
	6	12.94	5.47	24.5
	7	16.06	7.29	29.0
	8	19.72	9.44	34.2
	9	24.21	12.01	40.9
	10	30.11	15.21	50.2
	11	39.16	19.47	66.4
	12	100.00	26.06	—
30	0	0.00	—	2.0
	1	0.68	0.03	3.17
	2	1.37	0.24	4.25
	3	2.08	0.56	5.31
	4	2.82	0.96	6.35
	5	3.58	1.40	7.40
	6	4.36	1.89	8.45
	7	5.18	2.41	9.53
	8	6.01	2.98	10.63
	9	6.88	3.57	11.76
	10	7.79	4.20	12.92
	11	8.73	4.87	14.13
	12	9.71	5.58	15.38
	13	10.74	6.32	16.68
	14	11.81	7.11	18.04
	15	12.94	7.94	19.46
	16	14.14	8.82	20.96
	17	15.40	9.76	22.55
	18	16.74	10.76	24.24
	19	18.18	11.82	26.06
	20	19.73	12.96	28.01
	21	21.40	14.20	30.15
	22	23.23	15.53	32.49
	23	25.25	17.00	35.12
	24	27.52	18.62	38.10
	25	30.12	20.43	41.57
	26	33.17	22.50	45.78
	27	36.90	24.92	51.15
	28	41.82	27.86	58.73
	29	49.35	31.70	72.04
	30	100.00	37.54	—

$$E(\hat{U}) = \sum_{v=0}^{n} (v/n)^{1/k} \frac{n!}{v!\,(n-v)!} U^{kv} (1 - U^k)^{n-v}.$$

For various values of disease incidence, R, Table 2 illustrates the magnitude of the bias, $E(\hat{R}) - R$, for various numbers of groups. The estimate of R is expected to be an overestimate (this does not mean, of course, that any particular estimate exceeds the true value). The bias is small relative to the magnitude of incidence for low levels of disease incidence; it's importance will depend on the particular case.

Keeping the total sample size at 60, Table 2 also indicates the decrease in bias obtainable by increasing the number of

TABLE 2. Bias, $100(E(\hat{R}) - R)$, in estimating percentage disease incidence for groups of five fish each.

	Number of groups				
100(*R*)	12	30	60	100	300
90	9.99	9.98	9.97	9.96	9.90
80	19.77	19.52	19.16	18.74	17.05
70	28.25	26.42	23.94	21.24	12.52
60	32.87 (8.32)[a]	26.24	18.74	12.40	2.32
50	30.22	16.69	7.02	2.73	0.45
40	19.62	5.50	1.38	0.64	0.20
30	7.53	1.24	0.50	0.29	0.09
26	4.32	0.80	0.36	0.21	0.07
20	1.72 (0.45)[a]	0.47	0.23	0.13	0.04
16	0.98	0.33	0.16	0.09	0.03
12	0.60	0.22	0.11	0.06	0.02
8	0.35	0.13	0.06	0.04	0.01
4	0.15 (0.07)[a]	0.06	0.03	0.02	0.00
2	0.07	0.03	0.01	0.01	0.00

[a]Bias based on 20 groups of three fish each.

TABLE 3. Width of 90% confidence intervals for various percentage disease incidences (100*R*), number of individuals per group (*k*), and expected disease positive groups (*Ex*) observed from a total of 100 groups.

			90% confidence limits		
100*R*	*k*	*Ex*[a]	Lower	Upper	Width
10	5	41	7.6	12.8	5.2
	1	10	5.5	16.4	10.9
20	5	67	16.1	24.1	8.0
	1	20	13.7	27.7	14.0
30	5	83	24.6	35.5	10.9
	1	30	22.5	38.4	15.9
40	5	92	32.5	47.4	14.9
	1	40	31.8	48.7	16.9
50	5	97	40.3	61.7	21.4
	1	50	41.4	58.6	17.2
60	5	99	45.8	78.0	32.2
	1	60	51.3	68.2	16.9

[a]$Ex = 100(1 - (1 - R)^k)$.

groups and decreasing the number of individuals per group to 20 and 3, respectively. Again, in any particular case, improvement in the estimate would have to be considered in relation to the cost and time of analyzing additional samples.

Considering the bias and width of confidence intervals, the estimator is not very good for those cases in Table 1 where most of the groups are observed to be disease positive. Improved estimates require additional sampling.

Further, if disease incidence is large, pooling material from several fish may lead to less precise estimates than does making disease determinations on individuals (Table 3). For each hypothetical disease incidence considered, it is assumed that 100 groups of fish are analyzed; and that in one case each group is a single fish and in the other, a pool of five fish. For comparative purposes it is also assumed that the expected number of disease positive groups is observed in each case. Using the width of the 90% confidence interval to judge precision, this example indicates that for disease incidence up to about 20% there is a significant gain realized by pooling individuals into one sample. However, for 30% and above there is little to be gained or an actual loss in precision (Table 3).

Optimal group size, k', in terms of maximum precision can be examined in general by minimizing $V(\hat{R})$ (equation 6) over k for $k \geq 1$ and fixed n and U. This is most easily done by minimizing $(U^{-k} - 1)/k^2$ over k numerically since optimal k is independent of the value of n. For various values of R, pairs of $(100R,k')$ are: (1,159), (1.5,105), (2,79), (3,52), (5,31), (10,15), (20,7), (30,4), (40,3), (50,2), and ($\geq$ 70,1).

A limiting factor to group size is the ability to detect a diseased individual among a possible $k-1$ disease negative individuals in a group of size k. Another factor is the increased bias of $\hat{R}$ related to increasing group size for fixed n. For example if $R = 0.05$ and $n = 12$, the bias $(\hat{R} - 0.05)$ is 0.002, 0.003, 0.063, and 0.359 for k values of 5, 10, 31, and 50, respectively. While the optimum group size is 31 for an R of 0.05, repeated sampling of 12 groups of 31 individuals each produce estimates of R that vary around 0.113 (0.05 + 0.063) rather than 0.05.

Increasing the number of groups sampled decreases the bias. In the above example if k is held at its optimum value (i.e. $k = 31$), the bias $(\hat{R} - 0.05)$ is 0.063, 0.034, 0.013, 0.006, and 0.003 for n values of 12, 15, 20, 25, and 30, respectively. This example as well as others where the incidence level is as low as 0.01 indicate that a sample size (n) of 30 is sufficient to limit the bias to 0.003 when the optimum group size is used.

For practical purposes it appears that we can define a large sample size (n) as 30 so that equation 5 can be safely used for computing confidence intervals and for determining sample sizes ($\geq$30) needed to limit the width of those intervals to a desired value.

The problem addressed by Ossiander and Wedemeyer (1973) is the sample size in terms of individuals needed to detect a given disease incidence. When a disease positive group of one or more individuals is found, disease determinations can stop with reasonable assurance that the incidence level is at least the specified value. When all groups are disease negative it is unlikely that the incidence level is as great as the specified value. If a lower or upper bound is all that is needed for the problem at hand, their sampling scheme is the most economical. One needs only to determine a maximum group size such that a diseased individual can be detected.

Here we are concerned with examining the expected precision (width of confidence interval) in estimating disease incidence for various combinations of group size, k, and number of groups, n, sampled. One half the width of 90% confidence intervals, calculated from (5), are shown in Table 4 for values of k and n in which the bias $100(\hat{R} - R)$ is less than 0.5 and for incidence levels of 1, 2, 5, and 10%. In most cases the bias is $\leq$0.2 for $n \leq 30$ and $<$0.1 for $n > 30$.

If d_1 is a value in Table 4 corresponding to a disease incidence of say $100R_1$, and if the associated n_1 groups of k_1 fish each are sampled, we can assert with probability 0.90 that the estimate $100\hat{R}$ will be within d_1 units of $100R_1$. In this way, Table 4 provides some insight to the precision that can be expected for different sampling levels and group sizes

TABLE 4. One half width of 90% confidence intervals for disease incidence.

Number of groups (n)	Percent disease incidence ($100R$)															
	1.0				2.0				5.0				10.0			
	Group size (k)				Group size (k)				Group size (k)				Group size (k)			
	1	5	10	159	1	5	10	79	1	5	10	31	1	5	10	15
12	4.7	2.1	1.5	—	6.6	3.0	2.2	—	10.3	4.9	3.7	—	14.2	7.1	—	—
20	3.6	1.6	1.2	—	5.1	2.3	1.7	—	8.0	3.8	2.8	—	11.0	5.5	4.5	—
30	3.0	1.3	1.0	0.4	4.2	1.9	1.4	0.7	6.5	3.1	2.3	1.8	9.0	4.5	3.7	3.5
60	2.1	1.0	0.7	0.3	3.0	1.4	1.0	0.5	4.6	2.2	1.6	1.3	6.4	3.2	2.6	2.5
90	1.7	0.8	0.6	0.2	2.4	1.1	0.8	0.4	3.8	1.8	1.3	1.0	5.2	2.6	2.1	2.0
120	1.5	0.7	0.5	0.2	2.1	1.0	0.7	0.4	3.3	1.5	1.2	0.9	4.5	2.2	1.8	1.8
150	1.3	0.6	0.4	0.2	1.9	0.8	0.6	0.3	2.9	1.4	1.0	0.8	4.0	2.0	1.6	1.6
250	1.0	0.5	0.3	0.1	1.4	0.7	0.5	0.2	2.3	1.1	0.8	0.6	3.1	1.6	1.3	1.2
350	0.9	0.4	0.3	0.1	1.2	0.6	0.4	0.2	1.9	0.9	0.7	0.5	2.6	1.3	1.1	1.0
450	0.8	0.3	0.2	0.1	1.1	0.5	0.4	0.2	1.7	0.8	0.6	0.5	2.3	1.2	1.0	0.9

when planning a sampling program.

Equation 5 also provides a way to determine the necessary sampling levels, n, when a certain level of precision, d, is needed. This is given by:

$$(7) \quad n = [Z_a (1 - R)/dk]^2 [(1 - R)^{-k} - 1],$$

where Z_a is the value of the standard normal distribution for desired confidence $100(1-2a)\%$. The most economical (smallest) value of n is obtained by using the optimal group size, k', in equation 7. The value of the unknown disease incidence, R, is also needed to determine n. However, if some notion of the range of R is known, a conservative sample size (i.e. maximum) is obtained by using the upper limit of that range. This is illustrated in Table 4 by observing the decrease in interval width with decreasing R for fixed k.

While equations 5 and 7 are useful in planning a sampling program and do provide reasonable confidence limits under certain restrictions, those limits are symmetric about $\hat{R}$ and will always be less than the respective limits calculated from equations 3 and 4. For these reasons the latter method is superior in reporting results.

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Seasonal Variation in Weight-Specific Growth Rates, Feeding Rates, and Growth Efficiencies in *Microgadus tomcod*

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SALINAS, I. M., AND I. A. MCLAREN. 1983. Seasonal variation in weight-specific growth rates, feeding rates, and growth efficiencies in *Microgadus tomcod*. Can. J. Fish. Aquat. Sci. 40: 2197–2200.

Among *Microgadus tomcod* from Cumberland Basin, Bay of Fundy, exponents of the weight–length relationship (liver, gonads removed) ranged from 2.74 in April, after spawning, to 3.47 in September, after summer feeding. This implies that growth rate per unit of weight is seasonally higher in larger fish, although this diminishes on an annual basis after 2 yr in these short-lived (<4 yr) fish. In feeding experiments larger fish after spawning increased feeding (weight-specific ration declined with weight in September, not in April–May) and grew faster, using proportionately more food for growth (growth efficiency not correlated with weight in September, positively so in April–May).

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Chez des *Microgadus tomcod* provenant du bassin de Cumberland, dans la baie de Fundy, les exposants de la relation longueur–poids (foie et gonades enlevés) varient entre 2,74 en avril, après la ponte, et 3,47 en septembre, après la période d'alimentation estivale. Cela signifie que le taux de croissance par unité de poids est saisonnièrement plus élevé chez les grands poissons, bien qu'après 2 ans, ce taux diminue sur base annuelle chez ces poissons à courte durée de vie (<4 ans). Dans des expériences de nutrition, on a constaté que les grands poissons se nourrissent davantage après la ponte (la ration, pour un poids donné, diminue en fonction du poids en septembre, mais non en avril–mai) et ont une croissance plus rapide, utilisant proportionnellement plus de nourriture pour la croissance (corrélation nulle entre le rendement de croissance et le poids en septembre, corrélation positive en avril–mai).

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SOME fishes derive reproductive energy from somatic tissues (e.g. MacKinnon 1972; Iles 1974; Dawson and Grimm 1980), which partly explains their seasonal fluctuations in body weight. Experiments on feeding and metabolic processes, or growth efficiencies, have not generally addressed consequences of such seasonal variations in body condition, so characteristic of the temperate zone. Our study examines changes in length–weight relations, size-specific food consumption, and growth efficiency of Atlantic tomcod (*Microgadus tomcod*) between times of year when their body condition was at its highest and lowest.

Materials and methods — Specimens of *M. tomcod* were collected by trapnet around the middle of each month from April through December 1981 in the Cumberland Basin, Bay of Fundy. Gut contents of each fish were removed by incision of the tracts within 3 h of being taken from the nets. The fish were then taken in ice water to the laboratory and frozen (−30°C) for up to 3 wk. As we were interested in somatic growth patterns independent of any seasonal shifts of energy from liver to gonad, these were removed — as were otoliths for age determination. Specimens were then dried at 100°C and weighed to 0.1 g.

In April and September live specimens were captured and kept individually separated by fine-mesh netting in laboratory tanks at 8°C. After being starved for 7 d, to ensure complete evacuation of guts, each fish was weighed and then daily given a known wet weight of an excess of dead shrimp, *Crangon crangon*, a prey species that constitutes about 7% of the diet at certain times of year (Salinas 1980). Food intake was thus believed to be at satiation levels.

The fish were fed for 27 d in April–May, but the September experiment had to be stopped after 6 d because of rapid onset of a fin-rot disease. Although it shortened the experiment, the fin rot did not depress food intake on the last day of the experiment. Final weights were recorded several hours after the fish in each experiment had ceased defecating.

Because we are concerned with differences between large and small fish at the two seasons, no purpose is served by converting their common food source to dry-weight or energetic terms. Weight-specific daily growth rate was estimated from wet weights at the beginning (W_o) and end (W_t) of the experimental periods (t in days) as

$$(1)\quad G = \frac{W_t - W_o}{t(W_o + W_t)/2} \times 100.$$

Weight-specific daily ration (R_{max}) was estimated from total food intake (I) over the periods as

$$(2)\quad R_{max} = \frac{I}{t(W_o + W_t)/2} \times 100.$$

Growth efficiency (E) is

$$(3)\quad E = \frac{W_t - W_o}{I} = \frac{G}{R_{max}} \times 100.$$

Results — To assess growth with age, statistically adequate samples (>10) of the required successive age-classes were available only from November and December. Although asymptotic growth cannot be well specified in the short-lived *M. tomcod* (maximum age 3+ yr in our samples), relative growth rate clearly declines in the second year (Table 1).

Except during June and July, the exponents of weight vs. length on Table 2 were significantly different from 3 ($P < 0.05$ by t tests), with an annual average of 2.95. A similar seasonal pattern was described by Salinas (1980) for *M. tomcod* in the same location in 1979. A standard 175 mm fish was lightest (5.1 g) in April, after spawning, and heaviest (7.9 g) in September, after summer feeding.

Whereas weight-specific daily growth rate was not significantly related to mean body weight in the September experiment, it was positively so in April–May (Fig. 1). Weight-specific daily rations, on the other hand, were unrelated to weight in April–May, but decreased significantly with weight in September (Fig. 2).

It follows that growth efficiency (eq. 3) differed seasonally. It was positively related to mean body weight in April–May ($E = 11.7 + 0.205W$; $F_{1,17} = 25.89$, $P < 0.001$), but not in September ($E = 16.1 - 0.0306W$, $F_{1,15} = 1.89$, n.s.).

Discussion — The seasonal variation in body weight of *M. tomcod* in Cumberland Sound resembles the seasonal pattern of feeding intensity, which in turn is associated with

TABLE 1. Dry weights of bodies (minus liver, gonads, otoliths) of *M. tomcod* expressed as log$_e$ of weight in g to reduce disparity in variances and to demonstrate diminished relative growth rate with age.

		0+ yr old			1+ yr old			2+ yr old		
Month	Sex	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
November	♀	1.28	0.432	11	2.06	0.183	115	2.43	0.274	66
December	♀	0.93	0.583	21	1.85	0.204	20	2.31	0.259	37
November	♂	0.59	0.692	17	1.86	0.146	25	2.15	0.168	15

TABLE 2. Monthly changes in the values of the parameters of the equation $\ln W = \ln a + b \ln L$ for the somatic dry weight (W) in g, excluding the liver, of female *M. tomcod* at length (L) in mm.

Month	$\ln a$	SE $\ln a$	b	SE b	n
April	−12.55	0.364	2.74	0.072	60
May	−13.09	0.556	2.86	0.106	30
June	−13.06	0.697	2.88	0.134	30
July	−13.11	0.638	2.87	0.126	29
August	−14.56	0.357	3.22	0.071	37
September	−15.85	0.438	3.47	0.086	33
November	−12.74	0.361	2.85	0.074	79
December	−12.53	0.485	2.79	0.096	73

changes in abundance of the main prey (about 75% by weight), the amphipod *Corophium volutator* (Salinas 1980). Although in 1981 the rate of recovery of body condition was slower than in 1979 (Salinas 1980) or in 1980 (unpublished observations), the seasonal maxima and minima were similar in all 3 yr.

Even although feeding in nature continues, commencement of decline of body mass in October coincides with the onset of the reproductive cycle. This implies that food alone was not sufficient to meet energy demands of growth and maturation of gonads. Since livers were removed, it is clear that energy was transferred from the musculature during winter. Similar sequences of reduction in somatic weight and parallel increases in gonads have been reported for a number of species, including *Perca fluviatilis* (Le Cren 1951), *Rutilus rutilus* (Mann 1973), *Hippoglossoides platessoides* (Bagenal 1957), and *Clupea harengus* (McGurk et al. 1980).

In *M. tomcod* the exponent values of dry body weight vs. length ranged from 2.74 in April after spawning to 3.47 in autumn (Table 2). This suggests that growth rate per unit body weight during the period prior to translocation of somatic energy to gonads is higher among larger fish. The experiments showed that these larger fish after spawning indeed grew more rapidly, both through increasing their relative food intake and using their food more efficiently. Other authors have found that growth efficiencies among fish fed ad libitum are generally negatively related to weight (reviews by Paloheimo and Dickie 1966; Jones 1976). Generally such studies have not separated gonadal and somatic components and seasonal variation has not been explored.

The results have bearing on naive inference of metabolic rates from growth curves. In the simple Pütter–Bertalanffy model, anabolism is proportional to length^2 or weight$^{0.67}$, although other exponents are used in generalized versions (see Jones 1976). In *M. tomcod* the relationship of food uptake to body weight varied seasonally. However, at the same time assumptions of isometric growth were violated. Growth models may be rendered descriptively more accurate,

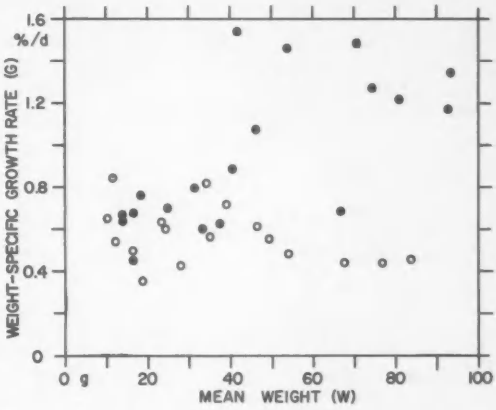

FIG. 1. Relationship between weight-specific growth rate (G, eq. 1 in text) and mean body weight (W) of *M. tomcod* in April–May (●, $G = 0.534 + 0.0092W$; $F_{1,17} = 16.300$, $P < 0.001$) and September (○, $G = 0.651 - 0.0023W$; $F_{1,15} = 2.526$, n.s.).

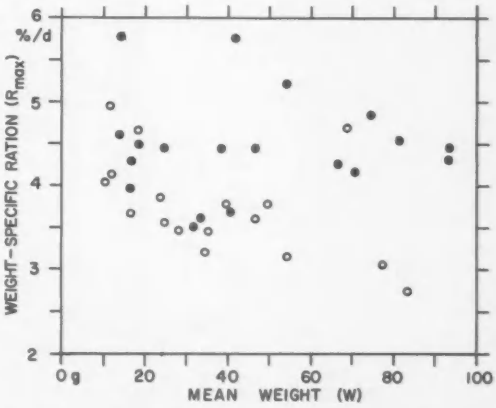

FIG. 2. Relationship between weight-specific daily ration (R_{max}, eq. 2 in text) and mean body weight in April–May (●, $R_{max} = 4.58 - 0.0012W$; $F_{1,17} = 0.056$, n.s.) and September (○, $R_{max} = 4.24 - 0.0132W$; $F_{1,15} = 5.042$, $P < 0.05$).

but not metabolically realistic, by introducing a seasonal fluctuation in gonad weight (e.g. Jones and Johnston 1977; Ursin 1979).

There remains the general question of how large fish can restore weight more rapidly despite overall slowing of growth on an annual basis. Part of the problem might disappear if growth is cast in "surplus-energy" form (Ware 1980). Also, while depleted reserves in fishes are not just fats, but also proteins, this is brought about by cell shrinkage rather than cell loss (review in Love 1980). In the related *Gadus morhua* the rate of increase of numbers of muscle cells decreases when the fish reach about 40 cm in length, after which there is substantial increase in cell diameters (Greer-Walker 1970). It may be that seasonal hyperplasia in older fishes is less "expensive" than the combined costs of cell division and metabolic storage in younger individuals.

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Counterimmunoelectrophoresis for Identification of Infectious Pancreatic Necrosis Virus After Isolation in Cell Culture

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An isolate of IPNV identified by seroneutralization test as serotype VR-299 was produced in RTG-2 cells, purified by density gradient centrifugation, and used to prepare the immune serum. Parameters of the CIE technique were adapted for the IPNV and one precipitin line was observed either with the purified antigen or with known positive specimens. A greater number of fish organ homogenates were positive after multiple passages in cell cultures than by direct CIE examination. However, the precipitin antigen was detected in cell culture fluid at early passages. The use of CIE following virus isolation in cell culture is recommended for routine identification of IPNV.

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Un produit isolé du virus de la nécrose pancréatique infectieuse (VNPI), identifié dans un test de séroneutralisation comme sérotype VR-299, a été formé dans des cellules RTG-2, purifié par centrifugation dans un gradient de densité et utilisé dans la préparation du sérum immunisé. La technique des paramètres de la CIE a été adaptée au VNPI et on a observé une ligne de précipitine, soit avec l'antigène purifié, soit avec des spécimens positifs connus. Un plus grand nombre d'homogénats d'organes de poissons sont positifs après de multiples passages en cultures cellulaires que par examen direct de la CIE. Cependant, l'antigène de précipitine a été détecté dès les premiers passages dans le liquide des cultures cellulaires. Nous recommandons, dans l'identification routinière du VNPI, d'utiliser la CIE après isolement du virus dans la culture de cellules.

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INFECTIOUS pancreatic necrosis (IPN) is a highly contagious and destructive disease that affects different species of fishes, particularly salmonids (Elazhary et al. 1976; Ahne 1978). The disease is characterized by rapid onset and high mortality in fry, fingerling and also in yearling brook trout (*Salvelinus fontinalis*) (Elazhary et al. 1976; Silim et al. 1982). The causative agent is a non-enveloped icosahedral virus, measuring 60–70 nm that possesses 92 capsomers, a double-stranded RNA, and has a density of 1.31–1.33 g/cm^3 in CsCl (Cohen et al. 1973; Dobos et al. 1977; Chang et al. 1978). The isolation of the virus from organs of infected fish in a variety of established fish cell lines, followed by a neutralization test with anti-IPNV serum, is still the most commonly used method of diagnosis (Malsberger and Cerini 1965; Wolf and Quimby 1967; Wolf 1970). Fluorescent antibody (FA) tests have also been employed for the detection of IPNV viral antigens in cell cultures (Piper et al. 1973; Tu et al. 1974) and in tissues from infected fish (Swanson and Gillespie 1981). The purpose of the present work is to describe and compare the use of counterimmunoelectrophoresis (CIE) to cell culture isolation for rapid diagnosis of IPNV.

Materials and Methods — Antiserum used in the CIE test was produced against a field IPNV isolated in our laboratory in 1978 and identified by serum neutralization test as reference strain VR299 (Silim et al. 1982).

Stock virus was prepared in continuous rainbow trout (*Salmo gairdneri*) gonad (RTG-2) cells propagated as monolayers in twenty 75 cm^2-Falcon tissue culture flasks as previously described (Silim et al. 1982). The cell cultures were infected at a multiplicity of infection of 0.1 $TCID_{50}$ (50% tissue culture infective dose) of virus per cell and incubated at 18°C until development of intensive CPE (cytopathic effect). Then they were harvested according to Chang et al. (1978) and the virus antigen was concentrated and purified by ultracentrifugation (108 000 × *g* for 2 h) on a three step CsCl density gradient [1.2, 1.3, 1.4 g/mL(w/v)]. The virus band was collected, dialyzed against TNE buffer and diluted to 5 mL in the same buffer. The purity of the viral antigen was checked by negative electron microscopy (EM) as described by Dea et al. (1979). The virus infectivity was titrated in 24-well Linbro tissue cultures plates according to the same authors. Titers were determined after a 5-d incubation period.

Antiserum to the concentrated virus was prepared from two New Zealand albino rabbits (2.5 kg). On the first day, they received an intravenous dose of 1.0 mL of purified IPNV antigen (viral suspension adjusted to $10^{7.0}$ $TCID_{50}$ per mL) and were also inoculated intramuscularly at four sites (0.5 mL per site) with a mixture of equal volumes of viral antigen and incomplete Freund adjuvant. On days 7, 14, and 21, they received only the intravenous dose. The animals were bled on day 30 and their serum was inactivated at 56°C for 30 min, diluted 1:5 in PBS solution and absorbed against RTG-2 cells to remove cellular antibodies. Titration of neutralizing activity was done as previously described (Dea et al. 1979) using RTG-2 cells prepared as for infectivity assay. The infected cultures were examined for evidence of CPE after an incubation period of 96 to 120 h at 18°C and the titer was expressed as the reciprocal of the highest serum dilution neutralizing 100 $TCID_{50}$ of virus.

For the CIE technique, tris-barbital buffer 0.05 mol/L (Bio-Rad Lab. Richmond, CA.) was used as tank buffer, and agarose was prepared in the same buffer diluted 1:2 with distilled water. Electrophoresis was carried out using a Gelman electrophoresis chamber (Gelman Ltd, Montreal, Canada) with the agarose (13 mL) on 8.3 × 10.2 cm Kodak projector slides. Two parallel rows of wells (4.0 mm in diameter) were punched at 0.7 cm center to center. Antigens and antiserum dilutions were prepared in the agarose buffer; cathodic wells were filled with 0.025 mL of antigen, and 0.025 mL of antiserum was poured in the anodic wells. A constant potential of 100 V was applied for times varying between 30 and 120 min. After electrophoresis, the slides were washed overnight in 0.85% NaCl and stained in 0.5% tannic acid as previously described (Dea et al. 1979).

The visceral organs from trout submitted to our laboratory for diagnosis of IPNV were pooled and homogenized in three volumes of Eagle's minimum essential medium (MEM). The suspensions (final dilution 1:4) were clarified by low-speed centrifugation and the supernatants were filtered through 0.22 μm Millipore filters (Millipore Co., Bedford, MA., USA). For each filtrate, two wells of a 24-well Linbro tissue cultures plates seeded with RTG-2 cells (70% confluent) were inoculated with 0.2 mL of filtrate and incubated at 18°C. The infected cultures were examined periodically for CPE and if none were recorded after 1 wk, cells were freezed-thawed

twice and the supernatants were used for two other blind passages.

Results — The purity of the antigenic preparation was confirmed by electron microscopy. It was then diluted 1:2 in 0.05 mol/L tris-barbital buffer and used to study the parameters of the CIE test. One precipitin line was observed between antibody and antigen wells, using as antibody the rabbit anti-IPNV prepared as in materials and methods (final dilution 1:5 before use).

No reaction was noted when normal rabbit serum was tested against the purified IPN viral antigen and with fish organ homogenates that were positive or negative in cell cultures. Non-infected RTG-2 cell extracts did not react with the antiserum, nor did cell cultures inoculated with negative fish organ homogenates. No precipitin line was observed between the anti-IPNV serum and the infectious haematopoietic necrosis virus (IHNV) antigen kindly furnished by Dr. Savan (OVC, Guelph University, Ont., Canada).

Electrophoresis was conducted at 200, 150, and 100 V for 30, 60, 90, and 120 min at 4°C. The best results were obtained with 100 V, and 60–90 min proved the most satisfactory. With this potential, the precipitin line did not migrate further towards the anodal well after 60 min and did not appear more distinct after 90 min.

A buffer of pH 8.0 gave better reactions than those of pH 7.5, 8.5, and 9.0. When antiserum and antigen wells were punched at 1.0 cm center to center, the results were not as clear as with a distance of 0.7 cm. The clearest reaction was also obtained by using agarose at a concentration of 0.7% instead of 0.5 and 1.0%.

Unstained, reacted agarose slides when viewed over a dark-field viewer generally showed weak precipitin reactions. Intensified antigen-antibody reactions within the agarose were obtained after immersing the slides for 20 min in 0.5% tannic acid after an overnight wash in normal saline following the electrophoresis. A longer exposure to tannic acid did not further amplify the precipitin line and higher concentrations of tannic acid gave excess background staining.

Tenfold dilutions (1:10 to 1:10 000) of the purified viral antigen prepared in 0.025 mol/L electrophoresis buffer were tested by CIE. The precipitin line could be easily visualized with antigen dilutions up to 1:20, but a weak reaction could still be noted at 1:100. The precipitin line was not observed when the antiserum was diluted more than 1:80. The endpoint dilution (1:100) of the virus preparation corresponded to $1 \times 10^{5.0}$ $TCTD_{50}$ per mL and the antiserum was shown to possess a neutralizing titer of 1:800. The experiment was repeated three times and identical results were obtained. After subjecting the viral antigen to three cycles of freezing and thawing, the precipitin reaction was still observed.

Eighty-five lots of fish submitted for diagnosis of IPNV were tested in parallel by cell culture technique and CIE. A clear CPE was produced in RTG-2 cells with 89.4% (76/85) of the organ homogenates while the IPNV precipitin antigen was detected by direct CIE in only 41.2% (35/85) of the cases. However, from the lots that were positive by the cell culture technique, only 27.0% (23/85) induced a CPE on their first passage, but 22 of 23 of these specimens were positive by direct CIE. From the specimens that induced CPE only after 2 (31/85) or 3 (22/85) blind passages on the cell monolayers, only 38.7% (12/31) and 4.5% (1/22) were detected by direct CIE, respectively, from the original homogenates. In case of cell cultures, the presence of IPNV was confirmed by neutralization of the CPE with the rabbit antiserum.

TABLE 1. Comparison between CIE and CPE results for the detection of IPNV in cell cultures inoculated with 45 clinical specimens.

Cell culture passage no.	CPE[a] No. + (%)	CIE[b] No. + (%)
1	3 (6.6)	21 (46.6)
2	5 (11.1)	26 (57.7)
3	26 (57.7)	26 (57.7)

[a]Presence of a cytopathic effect which was neutralized by the anti-IPNV serum.
[b]The CIE test was used to detect the IPNV precipitin antigen in infected cell cultures fluid.

A study was also done to determine the effeciency of the CIE test to detect the IPNV precipitin antigen in the culture fluid of RTG-2 cells inoculated with 45 other clinical specimens. After the first passage, 6.6% of the specimens tested induced a detectable CPE in cell cultures while the IPNV precipitin antigen was revealed in the supernatant fluid in 46.6% of the cases by CIE (Table 1). The number of positive lots by CIE (57.7%) was five times higher than detection of CPE after the second passage, and equal sensitivity was noted after the third passage. In most cases, staining in tannic acid was not necessary to detect the precipitin reaction.

Discussion — The CIE technique described was shown specific considering the results obtained with normal rabbit serum and the negative reactions noted when the anti-IPNV serum was tested against the IHNV antigen and non-infected cell extracts or fish organ homogenates. Only one precipitin line was observed in all the positive samples. Other authors have reported the presence of one or two precipitin antigens for other members of the Reoviridae depending of the degree of purification of the virus suspensions and the antiserum used (Bruggeman and Versteeg 1973; Middleton et al. 1976; Grauballe et al. 1977; Mohammed et al. 1978).

In this study, only a few specimens (41.2%) contained sufficient viral antigen to be revealed by direct CIE examination. A greater percentage of positive fish was demonstrated after isolation in cell culture monolayers (89.4%) but, in many cases, the CPE was observed after only two and three passages. This suggests that the original degree of infection of these fishes was lower than $10^{5.0}$ $TCID_{50}$ per mL which proved to be the limit of the CIE technique. On the other hand, the CIE test was very effective for detecting the IPNV antigen in infected cell culture monolayers. From the 26 specimens that were shown positive for CPE after three passages in cell cultures, 80.8% (21/26) were positive for the precipitin antigen in the culture fluid of first passage.

From these results, it appears that the sensitivity of the CIE test is probably not sufficient to detect the IPNV directly from tissues of carrier fish from which low virus titers are usually recovered, but the test could certainly be acceptable during acute outbreaks. However, the technique still offers a rapid

means of diagnosing IPN, following its isolation in cell cultures. The specificity of the test eliminates the need for many passages in cell cultures and the use of the seroneutralization test which is done to confirm the cell culture results.

Fluorescent microscopy is presently recognized as the most sensitive method for diagnostic of IPNV. Piper et al. (1973) reported that in RTG-2 cell cultures, the viral antigen could first be detected at 3 to 4 h post-infection when the virus titer had barely increased and was approximately 10^4 to 10^5 $TCID_{50}$ per mL. For this reason, Tu et al. (1974) suggested staining cells 6, 12, 24, 36, and 48 h after exposure which represents considerable work for routine diagnosis when many specimens are submitted. In a study using an indirect fluorescent antibody test for detecting IPNV in tissues of experimentally inoculated rainbow trout, Swanson and Gillespie (1981) easily recovered the virus from pancreas, liver and kidney, but in their experiment virus titers ranged from $10^{5.0}$ to $10^{7.5}$ $TCID_{50}$ per mL 10% tissue homogenates.

Further work is needed to compare and evaluate the sensitivity of these tests to CIE and to study the cross-reactivity of different IPNV strains using this test.

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The English–Wabigoon River System

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The English–Wabigoon River System: I. A Synthesis of Recent Research with a View towards Mercury Amelioration

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RUDD, J. W. M., M. A. TURNER, A. FURUTANI, A. L. SWICK, AND B. E. TOWNSEND. 1983. The English–Wabigoon River system: I. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. 40: 2206–2217.

Over a 3-yr period, teams from the federal Department of Fisheries and Oceans, the federal Department of the Environment, and the Ontario Ministry of the Environment researched the mercury pollution problem of the English–Wabigoon River system, northwestern Ontario, Canada. Mercury methylation and bioaccumulation were occurring mainly in the water column and surface layers of the sediments. Most mercury in the system was buried below the surficial sediments and probably does not contribute substantially to present mercury bioaccumulation. Movement of mercury between geochemical and biological compartments was rapid within the water column and surface sediments. Also, substantial amounts of inorganic and methyl mercury were transported down the river–lake system, having been released from stretches of river upstream of Clay Lake and deposited onto the surfaces of downstream lake sediments. Because of the constant movement of mercury into the water and onto downstream surface sediments, mercury amelioration procedures should concentrate on reducing bioaccumulation rates from both the water column and surface sediments. Treatments such as a single addition of clean clay to the surface sediments of lakes would probably be unsuccessful as long as significant downstream movement of mercury continues. Semicontinuous resuspension of bottom sediments followed by downstream deposition and/or elevation of the selenium concentration in biota by low-level additions of selenite were found to be the two most promising amelioration strategies. Both of these approaches would treat the problem in the water column and surface sediments. We consider advantages and disadvantages of these ideas and some needs for further research.

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Pendant une période de 3 ans, des équipes du ministère fédéral des Pêches et des Océans, du ministère fédéral de l'Environnement et du ministère ontarien de l'Environnement ont mené des recherches sur le problème de la contamination par le mercure dans le réseau des rivières English–Wabigoon, dans le nord-ouest de l'Ontario (Canada). La méthylation et la bioaccumulation du mercure se produit en grande partie dans la colonne d'eau et les couches superficielles des sédiments. La plus grande partie du mercure dans le réseau est enfouie sous les sédiments superficiels et ne contribue probablement pas de façon notable à la présente bioaccumulation de ce métal. Le mouvement de ce dernier entre les compartiments géochimiques et biologiques est rapide dans la colonne d'eau et dans les sédiments de la surface. Il y a en outre de substantielles quantités de mercure inorganique et de méthyl-mercure qui sont transportées en aval dans le réseau rivière–lac, ayant été mises en liberté dans des segments de la rivière en amont du lac Clay et déposées sur les surfaces des sédiments du lac en aval. À cause du mouvement continu du mercure dans l'eau et les sédiments superficiels d'aval, les méthodes d'amélioration du mercure devraient se concentrer sur la diminution des taux de bioaccumulation, tant dans la colone d'eau que dans les sédiments superficiels. Il est probable que des traitements tels que l'addition unique d'argile propre aux sédiments superficiels des lacs ne pourra réussir tant que le mouvement du mercure vers l'aval continuera à ce rythme. On a constaté que la resuspension semi-continue des sédiments du fond, suivie de la déposition en aval, et (ou) l'augmentation de la concentration de sélénium dans la biocénose par addition de bas niveaux de ce dernier sont les deux stratégies d'amélioration les plus prometteuses. Ces approches contribueraient toutes deux à la solution du problème, à la fois dans la colonne d'eau et dans les sédiments de surface. Nous examinons les avantages et les désavantages de ces options et le besoin de plus de recherche.

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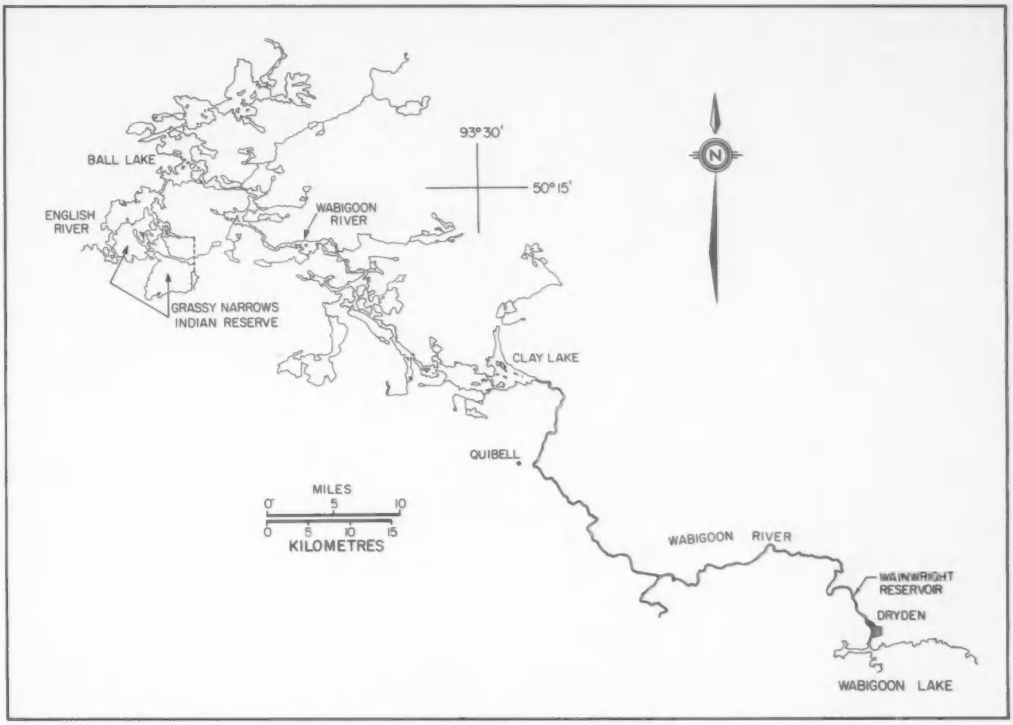

FIG. 1. The Wabigoon River system from Wabigoon Lake, northwestern Ontario, to the outflow of Ball Lake.

THE English–Wabigoon River system in northwestern Ontario is one of the most heavily Hg-contaminated waterways in Canada. The source of this Hg was a chlorine-alkali plant located in the town of Dryden. Armstrong and Hamilton (1973) estimated the discharge to have been between 9000 and 11 000 kg of mercury between 1962 and 1969. Since 1970, Hg discharges have been controlled and are reduced to about 1% of uncontrolled discharges (Parks et al. 1980). Despite this reduction of Hg discharge, the Hg concentrations of fish in some parts of the river system remain about 10 times above the legal limit for human consumption (e.g. Rudd et al. 1980b).

Wabigoon Lake, where the town of Dryden is located, is the headwater of the Wabigoon River (Fig. 1). The Wabigoon River system is joined by the English River system at Ball Lake. The combined river systems then flow into the Winnipeg River and then on into Lake Winnipeg. Deposits of glacial clay with rock outcrops are characteristic of the area. They are particularly common between Dryden and the outflow of Clay Lake, the first major downstream lake in the system. Erosion of these deposits gives the river–lake system a high turbidity, which tends to decrease with distance from Dryden (Parks et al. 1980; Rudd and Turner 1983a). This material, when it is either in suspension or in the surface sediments, is believed to have an important influence on rates of Hg bioaccumulation (Rudd and Turner 1983a).

The following is a synthesis of information on Hg pollution obtained by Federal (DFO and DOE) and Ontario (OME) Government research teams working on the English–Wabigoon River system since 1977 (Fig. 1). The overall objective of the three research teams was to understand Hg cycling in the system to a point where specific amelioration procedures could be recommended with reasonable confidence. The OME and DOE teams investigated the Hg problem by studying the English–Wabigoon system in its present state. They sampled sediment, water, and biota at various sites on the system from Wabigoon Lake to the Manitoba–Ontario border. Their studies were intended to provide a better understanding of the quantities and forms of Hg moving down the system, sediment–water–biota interactions, and seasonality of Hg movement. Clay Lake was sampled cooperatively by OME and DFO teams. Much of the DFO effort was expended on large ecosystem-level enclosure experiments and on direct assays of Hg methylation by natural microbial communities of the river system. These experiments were designed to provide a better understanding of the mechanisms of movement of Hg into biota and to identify key factors controlling the rate of Hg bioaccumulation by fish. The DFO team also tested certain proposed amelioration ideas. Preliminary results of these studies are available (Jackson and

Woychuk 1980; Parks et al. 1980; Rudd et al. 1980c).

To provide further insight into our experimental results and assist in the understanding of Hg cycling in the English–Wabigoon system we have also included discussion of recent research carried out on the Churchill–Nelson River system by another Freshwater Institute team headed by R. E. Hecky (unpublished data). This system is similar to the English–Wabigoon system in that it is also a river–lake system dominated by the deposits of glacial clay. It has recently developed a Hg pollution problem (Bodaly and Hecky 1979). Many of the methods used for the Churchill–Nelson study were similar to those used at Clay Lake, so that these two studies are directly comparable.

The comparative and experimental approaches of the research teams have resulted in information that is mutually supportive. When these results are viewed together, they provide a much improved understanding of Hg cycling in the system and point the way towards practicable amelioration procedures.

Methods

Samples for total Hg concentrations in sediments, water, and biota plus methyl mercury in water were taken on a fixed schedule. They were analyzed at the Freshwater Institute and by OME for total Hg concentration using atomic absorption spectrometry and for methyl mercury concentration by gas chromatography (Furutani and Rudd 1980; Jackson and Woychuk 1980; Parks et al. 1980). Data were interpreted by a mass balance approach in which partitioning of Hg between compartments (water, sediment, and biota) and the movement of Hg down the system were examined.

Several DFO experiments were carried out in 100-m^3 plastic walled enclosures located in Clay Lake. These enclosures contained complete ecosystems, including several species of small fish that grew on food produced in the enclosures during the experiments. Movement of Hg and Se through these ecosystems was followed radiochemically. Each experiment consisted of two control enclosures plus test enclosures perturbed in a controlled manner by the addition of nutrients (Rudd and Turner 1983b), bottom sediments (Rudd and Turner 1983a), Se (Turner and Rudd 1983; Turner and Swick 1983), or by the presence of a plastic bottom that isolated the water column from the sediments (Rudd et al. 1980b, 1980c; Rudd and Turner 1983b). These experiments were interpreted by comparing between the control and test enclosures the rates of movement of Hg and Se radioisotopes through the ecosystems and into biota.

Mercury-203 was added to the enclosures as Hg^{2+}, this being the chemical species that is both bacterially methylated (Jensen and Jernelov 1969) and is prevalent in the system (Parks 1976). Selenium-75 was added as selenite because it is the most probable chemical species to be added as a Hg ameliorating agent (Turner and Rudd 1983). The single-pulse addition of these chemical species, which were radioactively labeled, enabled us to follow the movement of the labeled atoms through the ecosystem and to determine the effect of an experimental manipulation (e.g. addition of Wabigoon Lake sediment) on their movement. The Hg and Se added are not assumed to have come into equilibrium with natural Hg and Se previously in the enclosures. Rather, we were breaking into the biogeochemical cycles of these elements by introducing the chemical species that were of particular importance to us. By using radioisotopes we were able to follow these additions throughout the cycle. Other advantages of using isotopes for these experiments were (1) analytical sensitivity of the radioassay methods minimized chemical additions required and thus minimized perturbation of the ecosystem and (2) a definite time zero for the beginning of the experiment could be established.

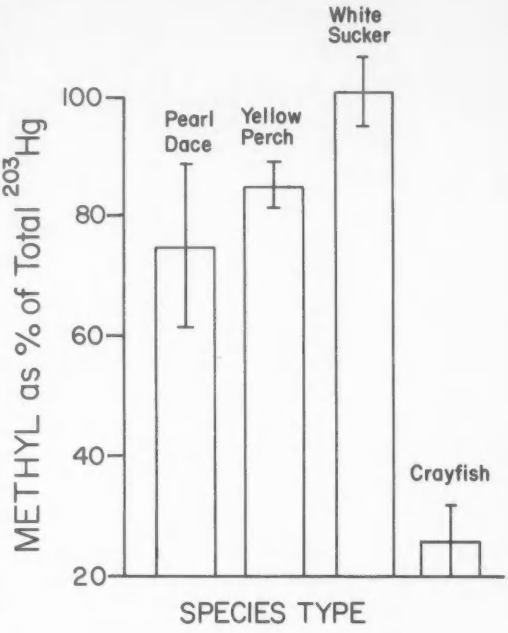

FIG. 2. An example of the proportion of the total Hg present as methyl mercury in muscle samples of fish and crayfish after 8 wk of exposure in a control enclosure. Standard deviations about the mean are shown (N = 3). The samples were first counted by gamma spectrometry to obtain estimates of total ^{203}Hg and then extracted for radiolabeled methyl mercury according to the technique of Uthe et al. (1972). Details of the method used are presented in Turner and Rudd (1983).

For certain experiments (Rudd et al. 1980b; Turner and Rudd 1983; Turner and Swick 1983), stable SeO_3^{2-} (at 1, 10, and 100 $\mu g \cdot L^{-1}$) was added to the enclosures. In these cases the ^{75}Se was used as a true radiotracer.

The enclosure experiments were intended to examine the effects of the experimental perturbations on methyl mercury bioaccumulation. On several occasions fish muscle samples were examined to ensure that the $^{203}Hg^{2+}$ was being methylated and bioaccumulated by the fish (Fig. 2).

In addition to the enclosure experiments, the DFO team developed a sensitive radiochemical method capable of measuring Hg methylating activity by natural microbial communities. Using this method the team found that Hg is methylated

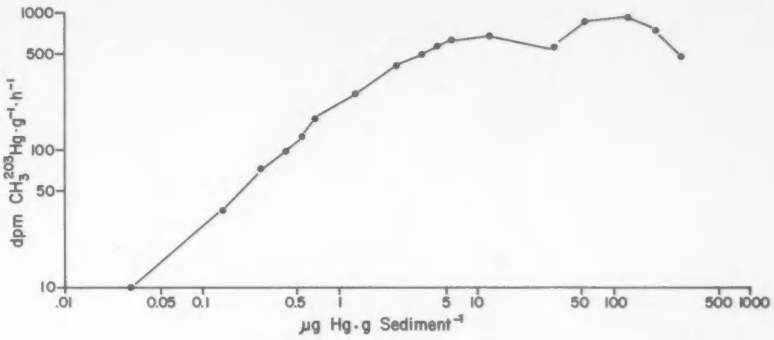

FIG. 3. Influence of inorganic Hg concentration on methyl mercury production rate. Aliquots of a surface sediment sample were incubated for 18 h in 250-mL ground glass stoppered bottles in the presence of increasing concentrations of $^{203}Hg^{2+}$. Analyses of the quantities of $CH_3^{203}Hg^+$ were carried out as described in Furutani and Rudd (1980).

in the water column and sediments (Furutani and Rudd 1980) and in the intestines of fish (Rudd et al. 1980a). Results of experiments using this technique at the Experimental Lakes Area, northwestern Ontario, are also discussed.

Results and Discussion

MERCURY CYCLING IN THE ENGLISH–WABIGOON RIVER SYSTEM

The compelling overall impression of the English–Wabigoon data is of the rapidity of Hg cycling within the river system and the responsiveness of this cycling to short-term physical and chemical changes. At all sampling sites, Parks et al. (1984) found a similar relationship between the concentration of Hg in the surface sediments and the concentration of Hg in the water column. Because this Hg partitioning was occurring in a flowing river system, this suggested that equilibrium was approached rapidly, i.e. within days. We also examined Hg partitioning within the water column of the enclosures. In less than 2 d, radiolabeled Hg had partitioned stably into five chemical fractions. Subsequently, the relative abundance of these fractions remained unchanged as Hg accumulated in the bottom sediments (Rudd et al. 1980b, 1980c; Rudd and Turner 1983a, 1983b; Turner and Rudd 1983). Residence time of the labeled Hg in the water column was quite short (about 20 d). These residence times are similar to those observed in both enclosure and whole lake additions of ^{203}Hg at the Experimental Lakes Area (Hesslein et al. 1980; Schindler et al. 1980). The estimates of Parks et al. (1984) on the speed of sediment–water partitioning of Hg in the polluted English–Wabigoon system resembled these radiochemical residence times.

As expected from such a rapidly cycling system, there was a quick (within days) response to changes in factors controlling the partitioning between various geochemical compartments. Parks et al. (1984) found that increases in water temperature were followed by increases in total Hg and methyl mercury concentrations in water, apparently as a result of desorption of Hg from sediments and/or increased methylation rates. We found that changing the chemical conditions in some enclosures, such as by the addition of 100 μg $Se \cdot L^{-1}$, increased the rate of movement of labeled Hg into the sediments (Rudd et al. 1980b; Turner and Rudd 1983). Resuspending clay-rich sediments in an enclosure had a much different effect, in that the Hg was immediately bound by the suspended sediments. Because the Hg-containing particles sedimented slowly, the residence time of the labeled Hg in the water column, which was probably controlled by adsorption rates to the sediments, was not altered by this treatment. However, movement of the Hg into the biological compartment (fish, crayfish, and zooplankton) was immediately reduced by a factor of 10 (Rudd and Turner 1983a).

These examples demonstrate that Hg in the English–Wabigoon River system moves rapidly among the geochemical compartments and responds quickly to physical and chemical changes. Further (see next paragraph), movement from geochemical to biological compartments was also responsive to perturbation. This is important from the point of view of Hg amelioration because it would require different strategies to treat a system that responded slowly.

Within the biological compartment, Hg concentrations in fish might be expected to respond to changes in the following general control factors: (1) the rate of supply of methyl mercury to fish including both the rate of microbial Hg methylation and the availability of methylated Hg for bioaccumulation, (2) the efficiency of Hg bioaccumulation by fish from food and water, and (3) fish growth rate. Mercury concentrations in fish have been thought to be negatively correlated with rate of growth. These three factors were investigated in this study with the following results.

Supply of methyl mercury to fish — Rates of methyl mercury production were found to be responsive to several factors. For instance, Hg methylating activity in surficial sediments was directly related to Hg concentration over several orders of magnitude (Fig. 3). This concurs with Parks et al. (1983) who also found that methyl mercury concentrations in

TABLE 1. Mercury methylation rates were fast when surface sediments from ELA lakes were incubated under nitrogen or under air but without shaking. When the same sediments were shaken to promote dissolution of oxygen from the air, methylation rates were much slower. One gram dry weight of sediment was incubated for 18 h at in situ temperature in a 250-mL ground glass stoppered bottle in the presence of 1 μCi of 10 μCi $^{203}Hg \cdot \mu g\ Hg^{-1}$ (1 Ci = 37 GBq). Analyses of $CH_3\ ^{203}Hg^+$ produced during incubations were done as described in Furutani and Rudd (1980).

Date (1980)	Lake	Incubation conditions: Static with air ($dpm \cdot g^{-1} \cdot h^{-1}$)	Shaken with air ($dpm \cdot g^{-1} \cdot h^{-1}$)	Shaken with nitrogen ($dpm \cdot g^{-1} \cdot h^{-1}$)
May 6	114	13 900	0	—
May 16	239	113 000	118	211 000
May 26	114	33 000	34	—
June 25	114	20 800	10 600	20 900
July 15	223	45	37	40
Sept. 6	114	439	—	1 120
Oct. 10	114	5 600	53	5 800

water and fish at a particular sampling site on the river system were related to local total Hg and methyl mercury concentrations in the nearby surface sediment. These observations suggest that methyl mercury production at a particular site was being strongly influenced by total Hg concentrations in local surface sediments.

Another controlling factor that can act with, or independently of, Hg concentration is the rate of microbial activity. It was found that methylation rates in the Clay Lake surficial sediments could be increased by three orders of magnitude in 2 d by the addition of bacterial food, which stimulated microbial activity (Furutani and Rudd 1980). Similarly, methylation rates in surface sediments of the Churchill–Nelson River system varied by three orders of magnitude depending on the presence or absence of natural decomposable organic material, which stimulated microbial methylating activity (R. E. Hecky, Freshwater Institute, Winnipeg, Man., personal communication). These widely varying rates appeared to be related primarily to differences in rates of microbial activity and not to total Hg concentration. At Southern Indian Lake, in northern Manitoba, Hecky also found that increasing microbial activity by the addition of *Sphagnum* moss to an enclosure increased the methylation of Hg in the water column and surface sediments. Ultimately this led to fish Hg concentrations in the *Sphagnum* addition enclosure that were 30 times higher than in the control enclosures. Similarly, at Clay Lake it was concluded that stimulation of primary productivity, which in turn increased the rate of supply of decomposable substrate to bacteria, caused increased methylation rates and an increased rate of supply of methyl mercury to fish (Rudd and Turner 1983b). It is also interesting that Clay Lake was the only lake of five lakes examined in northwestern Ontario that had a detectable methylation rate in the water column even though all five lakes had detectable rates in surficial sediments (Furutani and Rudd 1980; J. W. M. Rudd, unpublished data). Clay Lake was also the most productive of the five lakes (K. Mills, Freshwater Institute, Winnipeg, Man., personal communication).

Presence or absence of oxygen was also found to quickly affect methylation rates, which were usually found to be orders of magnitude higher in the absence of oxygen (Table 1). Similarly, Parks et al. (1984) found that methyl mercury concentration increased in the Wabigoon River upstream from Clay Lake during a period of anoxia.

Sulfide is thought to inhibit Hg methylation (Beijer and Jernelov 1979). However, even in the anoxic high-sulfide surface sediments of Clay Lake, bacteria methylated Hg as rapidly as in non-sulfide-containing oxidized sediments (Furutani and Rudd 1980). This lack of a sulfide effect was confirmed by progessive increases in methyl mercury concentrations with time in anoxic water overlying sulfide-containing sediments in the western basin of Clay Lake (Furutani and Rudd 1980; Parks et al. 1980). The reason for this may be that there was an excess of ferrous iron in the surface sediments, which bound sulfide as FeS and limited its effectiveness as a Hg-binding agent (Furutani and Rudd 1980).

The addition of uncontaminated sediments to an enclosure reduced the supply of Hg to fish and other biota by an order of magnitude (Rudd and Turner 1983a). This was probably caused by binding of inorganic Hg and/or methyl mercury to the suspended sediment making it less available for methylation and/or bioaccumulation from both the water column and surface sediments of the enclosure. At Southern Indian Lake, presence of clay-rich sediment in an enclosure reduced Hg accumulation in fish by a factor of three (R. E. Hecky, unpublished data). Both of these enclosure experiments confirm a caged fish experiment done at the inflow and outflow of Clay Lake (Rudd and Turner 1983a). During the fish exposure period, methyl mercury concentrations were higher at the outflow than at the inflow, even though total Hg was lower in concentration at the outflow. This corresponded with a lower suspended sediment load at the outflow, suggesting that inorganic Hg at the outflow was more available for methylation. Also, caged fish bioaccumulated Hg faster at the outflow than at the inflow (Rudd and Turner 1983a), suggesting that the Hg was more bioavailable at lower suspended sediment concentrations. In support of these observations Parks et al. (1980) and Jackson and Woychuk (1980) observed that both the ratio of total Hg to methyl mercury and the suspended sediment

the suspended sediment load decreased with distance from Dryden. All of these observations suggest that Hg methylation and bioaccumulation are inversely related to the amount of Hg-binding particulates.

Bioaccumulation efficiency — A second factor that controls fish Hg concentration is the efficiency with which fish bioaccumulate methylated Hg. We found that this was also quickly responsive to deliberate manipulation. For instance, within weeks of Se addition (1–100 $\mu g\ Se \cdot L^{-1}$) to the water column of enclosures, rates of Hg bioaccumulation by fish and other biota were detectably decreased (Rudd et al. 1980b; Turner and Rudd 1983; Turner and Swick 1983). It was found that the added Se had been quickly bioaccumulated by fish food sources and that the decrease in rate of fish Hg bioaccumulation (up to twofold) was related to the Se concentration of the fish food. Assimilation of Hg from Se-elevated food was found to be reduced (Turner and Swick 1983). Reduction of Hg bioaccumulation in the presence of elevated Se was also observed in crayfish and predatory insects (Rudd et al. 1980c; Turner and Rudd 1983; B. E. Townsend, unpublished data). A food chain enhancement response was also observed in which the Se effect was more pronounced in predatory fish than in other fish (Turner and Swick 1983). Microbial Hg methylation rates in surface sediment samples were not reduced by the Se concentrations used in these experiments (J. W. M. Rudd, unpublished data).

The efficiency of Hg bioaccumulation by fish may also be affected by two other conditions observed in the English–Wabigoon River system. It has previously been thought that the sole site of Hg methylation in lakes was in the sediment, which necessitates movement of the methyl mercury into water or food before it can be accumulated by fish. However, in addition to methylation in sediments, methylation was observed in the water column of Clay Lake (Furutani and Rudd 1980). This methylating activity was episodic, appearing to be positively related to rates of Hg methylation in the surface sediments. Methyl mercury produced in the water column would be immediately available for bioaccumulation either via the gills or after incorporation in food.

Methylation also occurred in the intestinal contents of all five fish species tested (Rudd et al. 1980a). The bacterial methylators may have been inhabiting the intestinal slime layer of the fish. They were found to be active in fish taken from both Hg-contaminated and uncontaminated lakes. De novo methyl mercury produced in the fish intestine would probably be more available for bioaccumulation than methyl mercury incorporated in fish food (Phillips and Gregory 1979; Rudd et al. 1980a) and so would increase the efficiency of methyl mercury bioaccumulation. These observations indicate that Hg is likely to be methylated in the aquatic environment wherever there is microbial activity. Consequently, mercury amelioration strategies should address methyl mercury production in the whole system and not just in the sediments.

Fish growth rate — The rate of fish growth is a third factor controlling Hg concentration of fish. It has been hypothesized that faster fish growth rates in contaminated aquatic systems of high primary productivity would decrease Hg concentration in fish by dilution of bioaccumulated Hg (Beijer and Jernelov 1979). Contrary to this hypothesis, we found that increasing the growth rate of fish by stimulation of primary productivity in enclosures resulted in substantially increased Hg concentrations, as compared with unfertilized control enclosures when the stimulation of primary production was not sufficient to elevate pH. In the enclosure with the highest primary productivity the resulting elevated pH appeared to retard the bioaccumulation of methyl mercury somewhat, so that the Hg concentrations in fish were similar to the controls even though fish growth had been substantially increased (Rudd and Turner 1983b). Apparently, microbial methyl mercury production in these enclosures was stimulated by the increased supply of food (decomposed plankton) as fast (high nutrient addition) or faster (low nutrient addition) than fish growth rates. In the latter case, this stimulation of methylation was sufficient to overcome dilution of Hg by the enhanced fish growth rates.

The examples presented above consistently demonstrate the rapidity of Hg cycling in the English–Wabigoon system. They also show that the rate of Hg movement among compartments within the ecosystem (and into fish) can be quickly altered. The key to a successful amelioration strategy is to understand this system to the extent that key processes responsible for elevated Hg concentrations in fish can be identified and manipulated in a predictable way. We consider that the rate of supply of methyl mercury to the fish was the most amenable to manipulation because we observed the largest changes (often orders of magnitude) in the shortest time periods (Furutani and Rudd 1980; Rudd and Turner 1983a; Fig. 3). Attempts at reducing the efficiency of Hg bioaccumulation by Se additions (Rudd et al. 1980b; Turner and Rudd 1983; Turner and Swick 1983) were also successful, although Hg concentrations of the biota tested were reduced less dramatically (by up to twofold). The attempt at reducing the Hg concentration of fish by increasing the fish growth rate had the opposite effect on Hg concentrations of fish and crayfish. This was probably because of off-setting changes within the ecosystem that resulted in a stimulation of Hg methylation as well as fish growth rates (Rudd and Turner 1983b). The addition of algal nutrients is therefore not recommended as a Hg amelioration strategy.

Methyl mercury in fish is excreted slowly (Huckabee et al. 1979). For example, Lockhart et al. (1972) observed half-times in northern pike (*Esox lucius*) of about 2 yr. Thus, changes in factors controlling rates of methyl mercury production and accumulation by fish would have a greater effect on younger, faster growing fish than on older, slower growing fish. For this reason an overall amelioration strategy should include treatment of one or more of the factors controlling production and accumulation plus removal of highly contaminated older fish.

Compartmentalization of Mercury Cycling

In our discussion of Hg cycling in the English–Wabigoon system we have dealt only with those parts of the cycle that are within the water column and near the sediment–water interface (compartments I and II of Fig. 4) because older deeper sediments below the mixing depth (compartment III of

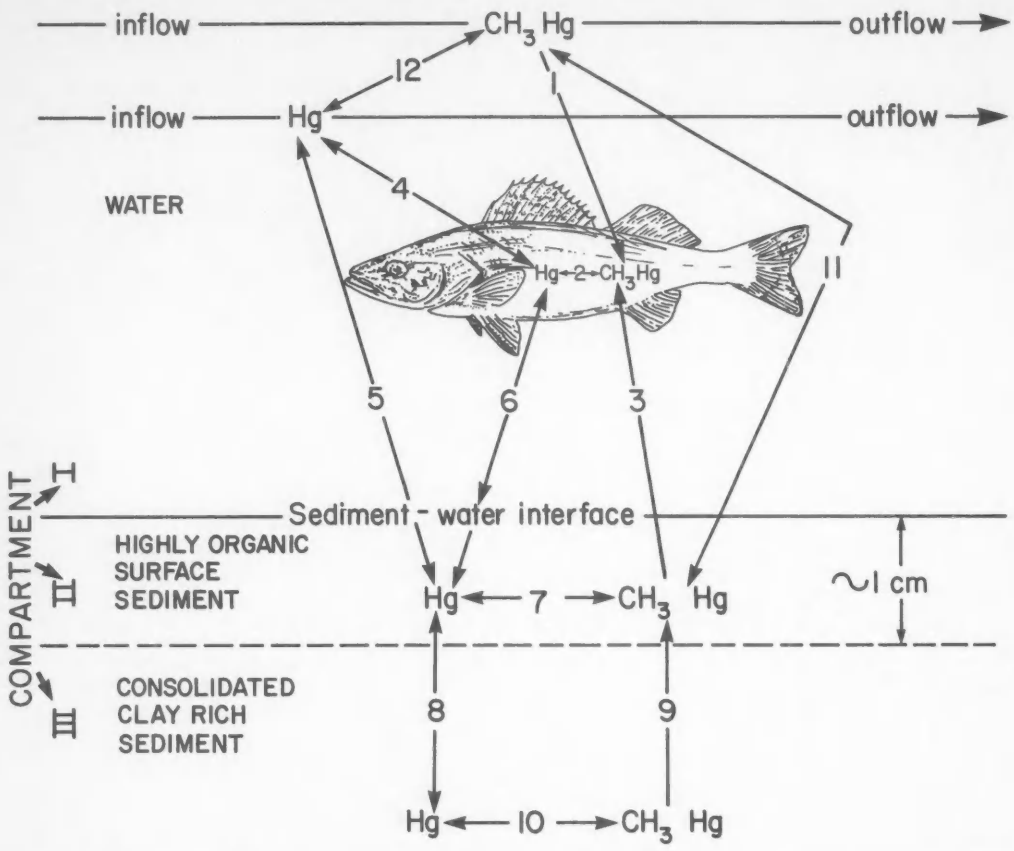

FIG. 4. A three-compartment model of Hg cycling in the English–Wabigoon River system showing interconnecting pathways of Hg movement from water and food into fish. Pathways 1, 2, and 3 are thought to be the major contributors of methyl mercury to fish. Deeper more consolidated clay-rich sediments (compartment III), which contain large quantities of Hg in some locations, do not contribute substantially to the present Hg pollution problem.

Fig. 4), which contain most of the Hg (Armstrong and Hamilton 1973; Jackson and Woychuk 1980; Parks et al. 1980), are not currently the most important sources of total Hg and methyl mercury to the biota. This conclusion is based on the following observations.

1) Locations on the Wabigoon River system that have large quantities of Hg in deep sediments (e.g. the Wainwright Dam and the eastern and western basins of Clay Lake) are not net sources of total or methyl mercury. In fact, Clay Lake is a net sink for total Hg (Parks et al. 1980).

2) Mercury currently being transported downstream to Clay Lake (Parks et al. 1980) originates from previously contaminated riverine surface sediments and from the pulp mill in Dryden and is sufficient to maintain present Hg concentrations of fish. Thus, there is no reason to think that movement of Hg from deep sediments contributes significantly to the current problem.

3) Parks et al. (1984) found that total Hg and methyl mercury concentrations in the water and biota at a particular sampling site were related to local Hg concentrations in the *surface* sediment.

4) Mercury concentrations of Clay Lake surface sediments have generally decreased since 1971 (Rudd et al. 1980c; Parks et al. 1984). Recent sediment profiles have peaks in Hg concentration up to several centimetres below the sediment–water interface (Rudd et al. 1980c), indicating that surface sediment concentrations have been gradually decreasing by deposition of cleaner sediments. Crayfish, which live at the sediment–water interface, have responded to this change with progressively decreasing Hg concentrations during the past few years (A. L. Hamilton, International Joint Commission, Ottawa, Ont., unpublished data). Mercury concentrations of fish also have been declining in recent years despite continued high Hg concentrations in deeper sediments of the

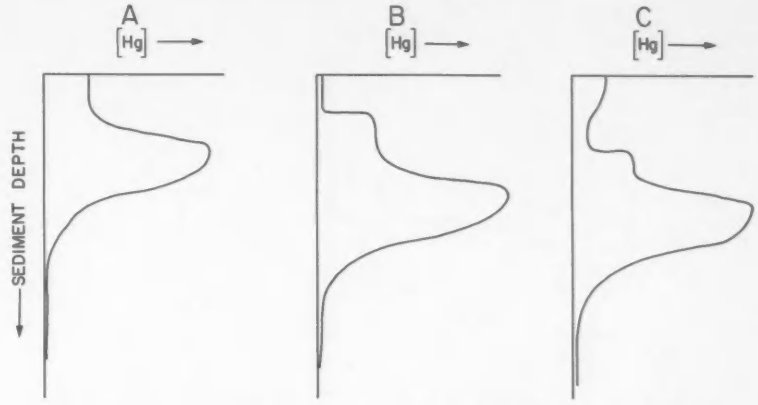

FIG. 5. (A) Schematic representation of a present Hg concentration profile in the English–Wabigoon River sediments; (B) profile after a single addition of clean clay; (C) shape of the profile several years after the clay addition after deposition of new contaminated surface sediment from upstream of Clay Lake resulting in a Hg pollution problem similar to the present situation. The precise shape of Hg profiles in Fig. 5B and 5C would be dependent on both rates of deposition of sediments and mixing depth.

system (Armstrong and Scott 1979).

5) Fish in an enclosure with a sealed plastic bottom containing compartments I and II of Fig. 4 (i.e. the water column and a layer of organic-rich material, which accumulated on the enclosure bottom during the experiment) bioaccumulated Hg more than 10 times faster than fish in a control enclosure with natural lake sediments (Rudd and Turner 1983a). We concluded that suspension of deeper clay-rich sediments and more rapid removal of Hg from the water by the clay-rich sediments (i.e. those in compartment III of Fig. 4) in the control enclosure reduced the rate of bioaccumulation by the mechanism discussed in Rudd and Turner (1983a). Similar observations have since been made in an enclosure experiment at Southern Indian Lake. Armstrong and Scott (1979) have suggested that suspended Hg concentrations might be an important factor controlling Hg concentrations of fish in Ball Lake (Fig. 1).

6) Using techniques adapted from those described in Furutani and Rudd (1980), rates of Hg methylation in sections of sediment cores were found to be highest near the sediment–water interface. Methylation rates decreased progressively with depth in the cores (J. W. M. Rudd, unpublished data).

These observations show that the present Hg contamination of fish in the English–Wabigoon system is not a consequence of Hg buried in the deep lake sediments. Instead, it is related to present water and surface sediment Hg concentrations. Surface sediment Hg concentrations may be maintained by erosion and desorption of Hg from upstream stretches of the river followed by downstream deposition onto lake surface sediments. In addition, there is still some Hg loading into the system from the pulp mill in Dryden (Parks et al. 1980).

Because Hg buried deep in lake sediments of the river–lake system is not the source of the present problem, it follows that this Hg should be left undisturbed. It also follows that amelioration procedures directed primarily towards Hg in deep lake sediments (such as dredging and harrowing), and which neglect present Hg loading to the water column and surface sediments, will probably be unsuccessful or even damaging.

FEASIBILITY OF SUGGESTED MERCURY AMELIORATION PROCEDURES

For the reasons discussed in the previous section, amelioration strategies such as the harrowing of lake sediments to dilute sediment Hg concentrations, or a single application of clean clay on the lake surfaces, would probably be unsuccessful in the long term if these were the only treatments undertaken. While short-term improvements might occur, an undesirable sequence would probably follow similar to that shown in Fig. 5 for a single clean clay application to the lake sediments. The present sediment Hg concentration profiles (Fig. 5A) that have a peak in concentration below the sediment–water interface would initially be favorably altered (Fig. 5B) by the reduction of surface Hg concentrations following application of clean clay. Concurrent transitory increases in suspended material would also be favorable (Rudd and Turner 1983a). However, continued erosion and desorption of Hg from surface sediments of upstream stretches of river followed by deposition onto the new clean clay of the surface sediments of the lakes would reelevate the Hg concentrations of the surface sediments of the lakes (Fig. 5C). After the reelevation of surface sediment Hg concentrations, improvement of the Hg pollution problem would probably follow a time course similar to the present rate of improvement.

Also, treating the system or specific sites with sulfide would probably not be successful. Sulfide could be effective in binding Hg in deep anoxic sediments but these sediments are not presently the problem. Treatment of surface sediments, which are continuously or periodically oxidized,

would be questionable because of sulfide oxidation. Further, even under anoxic conditions Hg methylation in surface sediment of Clay Lake was not inhibited by the presence of bound sulfide (Furutani and Rudd 1980).

Reduction of mercury loading — Further reductions of Hg loading into the system should improve the situation. Rates of Hg methylation in lake water and surface sediment are very responsive to changes in Hg concentration (e.g. Fig. 3). Recent further decreases in Hg discharge from the pulp mill in Dryden should help the problem (Parks et al. 1984). However, desorption and erosion of Hg from stretches of river, particularly between Dryden and Clay Lake (Fig. 1), continue to be a source of Hg to the river–lake system. Unlike dredging of lake sediments, removal of river sediments from the Wabigoon River between Dryden and Clay Lake is desirable although it is probably not economically feasible. Hydraulic flushing of the river stretch between Dryden and Clay Lake (Parks et al. 1980), followed by sedimentation in Clay Lake and Ball Lake, might be a possible method.

Selenium as a mercury ameliorating agent — The addition of Se (as selenite) to the river system was promising as a future ameliorating strategy. When 100 $\mu g\ Se \cdot L^{-1}$ was added to enclosures, the rate of accumulation of mercury by fish and other aquatic biota was reduced by a factor of two (Rudd et al. 1980b; Turner and Rudd 1983). The effect was both dose and species dependent. For example, white sucker (*Catostomus commersoni*) and yellow perch (*Perca flavescens*) were the only nonpredatory fish species that showed a significant reduction of Hg bioaccumulation at 1 $\mu g\ Se \cdot L^{-1}$ (Turner and Rudd 1983). The effect of Se was apparently transmitted via the food chain (Rudd et al. 1980b; Turner and Rudd 1983; Turner and Swick 1983). Food chain magnification of this Se effect was also observed (Turner and Swick 1983). Elevated concentrations of Se in water only were not effective at reducing Hg concentration (Turner and Swick 1983). In a laboratory investigation Klaverkamp et al. (1983a) may have observed a biphasic effect by waterborne Se. They found a reduction of methyl mercury bioaccumulation at 1 and 10 $\mu g\ Se \cdot L^{-1}$ but no apparent effect at 100 $\mu g\ Se \cdot L^{-1}$. When this information is considered in its entirety it suggests that long-term exposure of this river system to Se at concentrations below 10 $\mu g \cdot L^{-1}$ would probably reduce Hg concentrations in predatory fish.

Selenium is also known to reduce the toxicity of methyl mercury to fish consumers (e.g. Ganther and Sunde 1974). Concentrations of Se in Clay Lake fish are currently low (Turner and Rudd 1983). In our enclosure experiments, the added Se was accumulated very efficiently by fish (Rudd et al. 1980b, 1980c; Turner and Rudd 1983; Turner and Swick 1983) so that its addition would reduce the very high ratio of Hg:Se presently found in Clay Lake fish (Rudd et al. 1980b).

At the Se concentrations used in the enclosure experiments (1–100 $\mu g\ Se \cdot L^{-1}$) both of the ameliorative effects of Se are transmitted via the food chain (Rudd et al. 1980b; Turner and Swick 1983). This is an important advantage for Se as a Hg ameliorating agent because the beneficial effects of Se would be operating in the most active parts of the mercury cycle, i.e. the water column and surface sediments (compartments I and II of Fig. 4).

The possibility of Se toxicity to members of the food chain was also studied as part of our research (Klaverkamp et al. 1983a, 1983b; Turner and Rudd 1983; A. Salki, Freshwater Institute, Winnipeg, Man., unpublished data). A reported synergistic Hg–Se interaction in carp eggs (Huckabee and Griffith 1974) was not found in laboratory tests using rainbow trout (*Salmo gairdneri*) eggs (Klaverkamp et al. 1983b). Three species of juvenile fish had LC50 values for selenite that were at least four orders of magnitude above concentrations anticipated to be necessary for Hg amelioration (Klaverkamp et al. 1983a). No Se toxicity was found in the enclosure zooplankton communities at 1, 10, or 100 $\mu g\ Se \cdot L^{-1}$, and a shift in dominance of phytoplankton species occurred in enclosure experiments carried out at 10 and 100 $\mu g\ Se \cdot L^{-1}$ (A. Salki, unpublished data).

Despite the generally favorable results we are not yet recommending Se as a Hg ameliorating agent. Answers to several important questions are necessary before Se could be applied safely to the English–Wabigoon River system. These gaps in our knowledge are as follows.

1) The minimum water column and surface sediment concentrations that would be necessary for long-term improvement of Hg concentrations of fish are unknown. Our data suggest that concentrations below 10 $\mu g\ Se \cdot L^{-1}$ would be effective. However, we were unable to obtain more precise information because of the short duration of our enclosure experiments (up to 2 mo) and because it was difficult to maintain predaceous fish in the enclosure ecosystems. Until a minimum effective concentration is established the risk of Se intoxication of the most sensitive members of the food chain cannot be assessed. This question could be answered with a whole lake Se addition experiment in which movement as well as toxic and ameliorative effects of the added Se could be followed over several years in all levels of the food web.

2) At the present time it is not known how long a single Se addition would continue to be effective. Frequent small additions or a continuous slow rate of addition might prove to be the safest and most effective means of application because concentrations in water would be minimized.

3) If it was proposed that the river system be treated with selenite, possible toxic effects have not been adequately researched. Fish toxicity tests should be extended to include long-term chronic exposures of larval to adult fish (Klaverkamp et al. 1983b). A study of the minimum toxic concentrations to benthic insects, zooplankton, and phytoplankton species should be conducted because certain members of these communities have been found to be the most sensitive species tested.

4) Because microbial and chemical transformations of Se are known to occur in aquatic environments, production and toxicity of other Se species should also be studied (Klaverkamp et al. 1983a).

Resuspension and deposition of sediments as a mercury ameliorating procedure — In our experiments the largest reduction in rates of Hg bioaccumulation by fish and other biota was achieved by the addition of Wabigoon Lake sediments to an enclosure. This treatment reduced the rate of Hg bioaccumulation from the water column and surface sedi-

ments more than 10-fold (Rudd et al. 1980c; Rudd and Turner 1983a). A similar result has also been documented at Southern Indian Lake where rates of fish Hg bioaccumulation in enclosures with and without clay-rich sediments were compared (R. E. Hecky, unpublished data). Resuspension of uncontaminated sediments followed by downstream deposition is an attractive amelioration strategy because it attacks the Hg problem both in the water column and at the sediment–water interface.

Our sediment addition and resuspension experiments (Rudd and Turner 1983a) have directly demonstrated the water column and surface sediment effects. Research at Southern Indian Lake (R. E. Hecky, unpublished data) and the caged fish experiment carried out at the inflow and outflow of Clay Lake (Rudd and Turner 1983a) support these results.

Increasing the load of suspended sediment in the river system would also increase the rate of deposition of inorganic material onto the surface sediments. At the present time, Clay Lake sediments consist of an organic-rich surface layer, which overlies more consolidated clay-rich sediments. This increase in sedimentation rate of inorganic material would probably reduce Hg methylation in the surface sediments in two ways. It would dilute the concentration of recently deposited organic material in the surface sediments. Because the concentration of organic material can stimulate Hg methylation (Furutani and Rudd 1980; R. E. Hecky, unpublished data), in situ methylation rates would probably be reduced. In addition, we and others have demonstrated that methylation rates are a function of inorganic Hg concentration as well as other controlling factors (e.g. Fig. 3). Thus, dilution of sediment Hg concentration in surface sediments by increased deposition of clean clay would probably also reduce methylation rates. In support of this viewpoint, Parks et al. (1984) found that methyl mercury concentrations in both water and small fish are strongly related to Hg concentrations of the surface sediments at the same sampling site. For these reasons it is concluded that increasing the load of suspended sediments in the English–Wabigoon system would reduce the rate of movement of Hg into the biota from both the water column and the surface sediments.

Because there is presently continuous input of Hg to the surface sediments of the lakes in the system from desorption and erosion of upstream river sediments, frequent periods of sediment resuspension might be necessary (e.g. during each ice-free season). If periodic resuspension is not continued as long as Hg movement down the river continues, the result might be the unfavorable sequence presented in Fig. 5. We estimate that a few very small dredges (~3 t wet sediment per dredge hour) placed strategically along the river system would be sufficient to reduce the rate of Hg accumulation by the biota (Rudd et al. 1984). The number of resuspension sites would probably be governed by the importance of downstream mercury movement in determining Hg concentrations of surface sediments.

A first step in this amelioration approach would be the selection of suitable sources of material. Recent investigations at Southern Indian Lake (R. E. Hecky, unpublished data) have shown that clay-rich bank material obtained from above the water line had no effect on the rate of bioaccumulation of Hg when it was added to the water column of an enclosure. However, resuspension of sediments from the bottom of another enclosure at Southern Indian Lake had the same type of beneficial effect that we found at Clay Lake using sediments taken from the bottom of Wabigoon Lake. Thus, the source of this material appears to be crucial. Our present hypothesis is that differences in the affinity of Hg for the materials were caused by differences either in organic content of the substrate or in the iron or organic coating of the clay particles. The chemical differences among these materials are presently under investigation. In any case it would be prudent to test the efficacy of the material before large-scale resuspension commenced.

At the present time the resuspension and downstream deposition of clay-rich lacustrine sediments also appears to be a practical ameliorating strategy because there is an abundance of easily accessible material within the river system. Most likely sources include the sediments of Wabigoon and Eagle lakes and the sediments of the eastern or western basins of Clay Lake and Ball Lake (Fig. 1) although sediments of the latter lakes are partially mercury contaminated. The river basin between Dryden and Clay Lake also contains deposits of clay-rich sediment, which might be resuspended by diversion of the river within its channel or by removal of bed rock sills within this stretch of the river, as suggested by Parks et al. (1980).

Several important questions remain to be answered before sediment resuspension could be undertaken. (1) The concentration of resuspended sediment in water and surface sediments required to reduce Hg concentrations in water and biota to acceptable levels should be determined. (2) The relative importance of Hg bioaccumulation from the water column and surface sediments by fish should be assessed. For example, if surface sediments were substantially more important for fish Hg bioaccumulation, it might not be necessary to constantly maintain a high suspended load in the water column. (3) Testing the ameliorative efficiency of various sediment sources should also be undertaken. (4) The risks involved in resuspension of various ratios of clean and Hg-contaminated sediments should be assessed. (5) Moreover, the effects on primary productivity and fish growth rates in the system need to be more fully investigated. A study of the effects of increased suspended sediment concentration on fish productivity in Southern Indian lake will give us some information on the effects that resuspension of sediment could have on fish productivity in the English–Wabigoon River system (R. E. Hecky, unpublished data).

In summary, available data indicates that 9 yr after discharge of Hg into the English–Wabigoon River system was controlled, most of the Hg is buried in the sediments to a depth of greater than about 2 cm. This Hg is currently not an important source of Hg to the biota. Mercury presently in the water column and in the surface sediments is very reactive biologically, being methylated in the water column, in the intestinal contents of fish, and in the surface sediments. This methylating activity is thought to be responsible for the continuing high Hg concentrations in biota of the river system. Methylation and bioaccumulation of Hg are very responsive to imposed changes in certain chemical and biological parameters. Within days, elevated concentrations of suspended sediment or Se substantially reduced bioaccumulation of methyl mercury by fish while stimulation of primary productivity increased the methyl mercury concentration of fish. Based on

our present knowledge of Hg dynamics in the river system we recommend that a Hg amelioration procedure should focus on control of Hg methylation and bioaccumulation from both the water column and surface sediments. Mercury buried deeper in the sediments should be left undisturbed. The most promising amelioration approach would be a combination of procedures including resuspension of clay-rich sediments, plus further reduction in Hg loading into the system from both the pulp and paper mill and river surface sediments between Dryden and Clay Lake.

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The English–Wabigoon River System: II. Suppression of Mercury and Selenium Bioaccumulation by Suspended and Bottom Sediments

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Bioaccumulation of ^{203}Hg and ^{75}Se by several members of the food chain, including fish, was followed in large in situ enclosures in the presence and absence of organic-poor sediment. When the sediment was absent, ^{203}Hg was bioaccumulated 8- to 16-fold faster than when it was either suspended in the water or present on the bottom of the enclosures. Mercury-contaminated and uncontaminated sediments were equally effective at reducing the rate of radiolabeled mercury bioaccumulation, apparently by binding the mercury to fine particulates making it less available for methylation and/or bioaccumulation. Based on these results, a mercury ameliorating procedure involving semicontinuous resuspension of organic-poor sediments with downstream deposition onto surface sediments is suggested. The presence of sediments, in the water or on the bottom of enclosures, also reduced radiolabeled selenium bioaccumulation. The degree of inhibition (2- to 10-fold) may have been related to the concentration of organic material in the predominantly inorganic sediments. Implications of this research with respect to mercury–selenium interactions in aquatic ecosystems are discussed.

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Nous avons suivi la bioaccumulation de ^{203}Hg et de ^{75}Se dans plusieurs membres de la chaîne alimentaire, y compris les poissons, placés dans de grandes enceintes in situ, en présence et en l'absence de sédiments pauvres en matière organique. En l'absence de sédiments, ^{203}Hg est bioaccumulé de 8 à 16 fois plus rapidement que quand il est soit suspendu dans l'eau ou présent sur le fond des enceintes. Les sédiments contaminés par le mercure et non contaminés sont tout aussi efficaces à réduire le taux de bioaccumulation de mercure radioactif, apparemment par fixation du mercure sur de fines particules, ce qui le rend moins accessible à la méthylation ou à la bioaccumulation, ou aux deux. En nous fondant sur ces résultats, nous suggérons une méthode d'amélioration du mercure, impliquant une resuspension semi-continue des sédiments pauvres en matière organique, avec déposition en aval sur les sédiments superficiels. La présence de sédiments dans l'eau ou sur le fond des enceintes réduit également la bioaccumulation de sélénium radioactif. Il se peut que le degré d'inhibition soit relié à la concentration du matériel organique dans les sédiments en grande partie inorganiques. Nous analysons les implications de cette recherche sous l'angle des interactions mercure–sélénium dans les écosystèmes aquatiques.

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SUSPENDED sediments are thought to play an important role in the bioconcentration of toxic substances (Gibbs 1973; Hem 1976; Karickhoff and Brown 1978; Popp and Laquer 1980; Tessier et al. 1980). They have often been considered as vectors capable of moving pollutants from their sources to distant locations where the toxic substances can be accumulated by aquatic biota. At neutral pH the sediment-borne pollutants may be associated with particulate or dissolved organic materials, clay mineral, or organic and inorganic (usually Fe and Mn) coatings on the surfaces of clay particles (Andersson 1970, cited in Andersson 1979; Jonasson 1970; Hem 1972; Jackson et al. 1978; Andersson 1979; Jackson 1979).

Sediments might also serve a beneficial role by reducing the bioavailability of pollutants such as Hg. In the Hg-polluted English–Wabigoon River system (Armstrong and Hamilton 1973), the two most important sites of Hg methylation and bioaccumulation are the water column and surface sediments (Rudd et al. 1983). If sediments were deliberately resuspended as a Hg ameliorating procedure, they might reduce Hg methylation and bioaccumulation while the sedi-

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TABLE 1. Chronology of 1979 enclosure additions.

Days after isotope addition	Addition
−12 to −10	Enclosures filled
−10	3 kg NaCl per enclosure yielding final concentrations of 13 mg $Na \cdot L^{-1}$
−3	18 kg sediment added to the sediment addition enclosure
0	5.7 mCi $^{203}Hg(NO_3)_2$ and 1.4 mCi $H_2{}^{75}SeO_3$ to each enclosure on June 4, 1979
4	60 dace per enclosure at an initial density of 40 $kg \cdot ha^{-1}$
7	12 kg sediment added to the sediment addition enclosure
7	18 caged crayfish to each enclosure
9	6 caged clams per enclosure
22	12 kg sediment added to the sediment addition enclosure
30	Fourth sediment addition to examine short-term radioisotope speciation
30	Termination of experiment

TABLE 2. Wabigoon Lake sediments taken from the central basin of Wabigoon Lake. Surface organic floc was removed during sampling. All analyses are presented on a dry weight basis.

Sample	% sand < 50 μm	% silt 50−2 μm	% clay < 2 μm	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Carbonate (%)	Total Hg ($\mu g \cdot g^{-1}$)
Wabigoon Lake (as % of inorganic fraction)	9	46	42	—	—	—	—	—
Wabigoon Lake (as % of total sediment)	9	48	41	2.0	0.2	0.07	0.3	0.03

ments were suspended in the water column and after they were deposited onto the surface sediments. This possibility was examined during 1978 and 1979 using ecosystem-level experiments in large enclosures located in Clay Lake (50°03′N, 93°30′W), which is the first major reservoir of the English–Wabigoon River system. The experiments were designed to test the effects of sediment suspension on bioaccumulation of Hg by fish and other members of the food chain.

The effect of sediments on rates of Se bioaccumulation was also tested. This was done because trace Se addition to Hg-contaminated waterways was being considered as an ameliorating procedure (Rudd et al. 1980c; Rudd et al. 1983; Turner and Rudd 1983).

Methods

Two separate experiments were conducted in large enclosures during the summers of 1978 and 1979. The enclosures were 10 m in diameter and either 2 or 1.5 m deep with a volume of about 100 m^3. They were constructed of cross-laminated polyethylene as described in Rudd et al. (1980b, 1980c) except that most of the enclosures had watertight polyethylene bottoms. They were situated in a sheltered bay of Clay Lake, northwestern Ontario (Armstrong and Hamilton 1973). The plastic bottom enclosures were filled over a 3-d period with Clay Lake epilimnion water. Pumps were moved repeatedly from enclosure to enclosure to ensure that water and plankton in all the enclosures were similar.

The 1978 experiment had a control enclosure with a bottom of natural Clay Lake sediments and a test enclosure with a water-tight polyethylene bottom, which prevented contact with lake sediments. The 1979 experiment had three enclosures with sealed plastic bottoms. Sediment was added to the water column of one enclosure (sediment addition enclosure), while the two other enclosures were maintained as controls.

ENCLOSURE TREATMENTS

On day 0 of the experiments (July 15, 1978; June 4, 1979) each enclosure received approximately 5.7 mCi of $^{203}Hg(NO_3)_2$ (1 Ci = 37 GBq, approximately 15 ng $Hg \cdot L^{-1}$ was added as carrier) and about 1.4 mCi of $H_2{}^{75}SeO_3$ (New England Nuclear). A single addition of foodgrade NaCl was made to each enclosure for checks of enclosure leakage. The resulting Na^+ and Cl^- concentrations were approximately twice preaddition levels, as determined by atomic absorption spectrometry (Stainton et al. 1977). No leakage was detected during the course of either of the experiments.

The control enclosures received no other chemical additions. The 1978 plastic bottom enclosure received sufficient NaH_2PO_4 and $NaNO_3$ to maintain primary productivity rates at the level of the control. For the 1979 experiment, sediment was obtained from the central basin of Wabigoon Lake, which was upstream of Clay Lake and was not Hg contaminated. The surface layer (0.5 cm deep) of recently deposited organic material was removed. Sediment was added to this enclosure on three occasions (Table 1). The first addition was approximately 18 kg (dry wt) of Wabigoon Lake sediment while the second and third additions were about 12 kg each. On each occasion, wet sediment (59% water) was suspended throughout the water column of the suspended sediment enclosure by dispersing it into the wake of an electric outboard motor. Sediment addition continued until the Secchi depth (Welch 1948) was 20 cm. Particle size analysis of the sediment was done by the pipette method (McKeague 1978). This material was largely composed of silt and clay-sized particles and was of a low Hg concentration (Table 2). The sediment additions

TABLE 3. Secchi depth, total suspended solids, and Hg data at four sites on Clay Lake during the open water season (May–Nov.) of 1978.

Site	Secchi depth (m)	Suspended solids ($mg \cdot L^{-1}$)	Total Hg ($ng \cdot L^{-1}$)	CH_3Hg^+ ($ng \cdot L^{-1}$)
Inflow	0.58±0.10, $N = 24$	10.4±2.1, $N = 12$	35.3±10.7, $N = 48$	0.048 ($\div\times$1.69),[c] $N = 22$
	(0.54±0.07, $N = 9$)[a]	(9.2±0.8, $N = 5$)[b]		
Eastern basin	0.63±0.14, $N = 24$	8.5±3.5, $N = 12$	30.5±14.0, $N = 46$	
Western basin	1.27±0.42, $N = 23$	2.3±0.9, $N = 12$	19.6± 9.9, $N = 35$	
Outflow	1.39±0.42, $N = 24$	2.4±0.8, $N = 12$	17.9± 7.4, $N = 50$	0.10 ($\div\times$1.62),[c] $N = 22$
	(1.29±0.29, $N = 9$)[a]	(2.4±0.9, $N = 5$)[b]		

[a]Sampled weekly during Aug. 23–Oct. 18.
[b]Sampled biweekly during Aug. 30–Oct. 25.
[c]One standard deviation is obtained by multiplying or dividing the log-normal mean by the factor given in parentheses.

did not detectably increase Hg concentrations in the water of the enclosures.

RADIOACTIVE SAMPLE ANALYSES

The Hg isotope was added as $^{203}Hg^{2+}$ and Se was added as $^{75}SeO_3^{2-}$ (Table 1). A comparative radiochemical approach was used for this experiment, as opposed to a radiotracer approach. This was done by adding identical quantities of the radioisotopes to each enclosure. Bioaccumulation of the radioisotopes could then be compared between control and test enclosures. Further details of the radioisotope rationale are presented in Rudd et al. (1983).

Water samples were analyzed for ^{203}Hg and ^{75}Se speciation as in Hesslein et al. (1980) and Rudd et al. (1980b). The particulate fraction was obtained by sequential filtration through GF/C-Whatman and 0.45-μm Millipore filters. The filtrate was then passed through an activated charcoal column to obtain the fraction associated with organic material. This was followed by passage through a mixed cation–anion exchange column (Dowex 1 × 8, 100–200 mesh and 50W-12, 100–200 mesh) to remove the ionic fraction of the radioisotopes. Finally, any remaining or unassociated ^{203}Hg was precipitated as ^{203}HgS after oxidation to $^{203}Hg^{2+}$, as described in Rudd et al. (1980b).

Bioaccumulation of radioisotopes was monitored in crayfish (*Orconectes virilis*) and clams (*Anodonta* sp.) caged individually in epoxy-coated wire mesh cages and in free-swimming pearl dace (*Semotilus margarita*). The crayfish were fed an excess of white sucker (*Catastomus commersoni*) flesh, which had a low mercury concentration of 0.2 $\mu g \cdot g^{-1}$.

Five or six clams, crayfish, and pearl dace from each enclosure were analyzed on each sampling date. All samples for ^{203}Hg and ^{75}Se analyses were contained in small petri dishes (50 mm in diameter and 9 mm in height) and assayed by gamma spectroscopy (Hesslein et al. 1980; Rudd et al. 1980b). Sample means were calculated from the log transformed data, which had been decay corrected to day 0 of the experiment.

Zooplankton samples (i.e. particulate material greater than 73 μm) were collected using a plexiglass trap (Schindler 1969). For each sampling day, the contents of traps from 0.25, 0.75, and 1.25 m of depth were combined, dried at 60°C, and weighed before radioisotope analyses.

Movement of ^{203}Hg and ^{75}Se into the periphyton community was followed by assaying the periphyton colonized on polyethylene strips hung from the lake surface to a depth of 1 m in the enclosures. After assay of its radioisotope concentration, the periphyton was dried at 60°C, scraped from the polyethylene, and weighed. These strips were uncolonized when they were suspended in the enclosures at the time of enclosure installation. All radioisotope data were decay corrected to the day of radioisotope addition, day zero of the experiment.

The radioisotope bioaccumulation data are presented as ratios in which the lognormal means of the radioisotope concentrations (cpm per gram wet weight) of the test samples were divided by the means of the control concentrations.

OTHER ANALYSES

During 1979, weekly estimates of cloudless primary productivity were made using the ^{14}C incubator technique as described in Rudd and Turner (1983).

Total Hg and methyl mercury concentrations in water were analyzed according to methods described in Furutani and Rudd (1980). Total suspended solids were estimated as described in Stainton et al. (1977) except that samples were filtered with 0.45-μm membrane filters instead of glass fiber filters. Water samples were taken at 2-wk intervals and analyzed for total dissolved phosphorus, particulate carbon and phosphorus, and dissolved organic carbon, as described by Stainton et al. (1977).

CAGED FISH EXPERIMENT

Finescale dace (*Chrosomus neogaerus*) and pearl dace were held for up to 8 wk in 1-m^3 braided nylon cages suspended above the sediments at both the outflow and inflow of Clay Lake. Four individuals of each species were sampled by dip net on each sampling occasion and were frozen until analyzed for Hg concentration by the method of Armstrong and Uthe (1971).

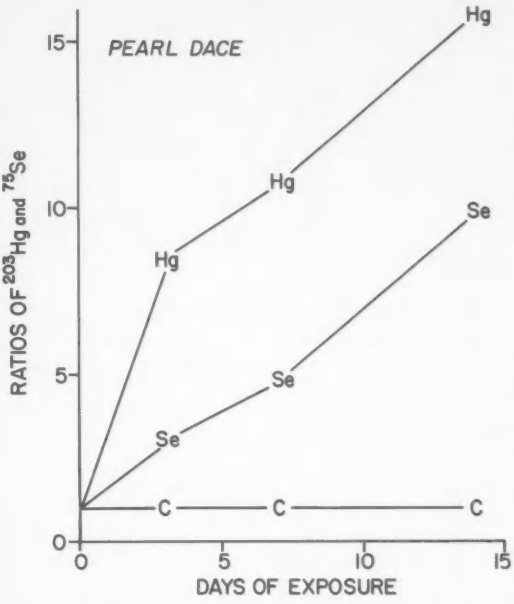

FIG. 1. Inhibition by natural lake sediments of pearl dace ^{203}Hg and ^{75}Se bioaccumulation expressed as ratios in which ^{203}Hg and ^{75}Se concentrations of fish in an enclosure with a plastic bottom are divided by corresponding concentrations in fish maintained in an enclosure with natural lake sediments (C).

Results

1978 EXPERIMENT

Wave action on the walls of the enclosures continually resuspended bottom sediments in the control enclosure maintaining the Secchi depth within a range of 0.7−1.0 m. This Secchi depth was close to the average Secchi depth of the eastern basin of Clay Lake where the enclosures were located (Table 3). Within 2 wk, Secchi depth in the enclosure with a sealed plastic bottom was greater than the depth of the control enclosure (2 m) and remained at greater than 2 m depth for the duration of the experiment (56 d). The water column residence time of ^{203}Hg in the enclosure with a plastic bottom was twice as long as in the control enclosure (36 vs 18 d) (Rudd et al. 1980c).

Radiolabeled Hg was bioaccumulated by pearl dace 8−16 times faster in the plastic bottom enclosure than in the control enclosure, which had natural sediments and more suspended particulate material (Fig. 1). At the end of the experiment ^{203}Hg concentrations in crayfish were 15 times higher in the plastic bottom enclosure than in the control enclosure (data not shown, the percent coefficient of variation of each mean concentration being about 20%).

Selenium-75 bioaccumulation was also reduced by the presence of sediments. Concentrations in pearl dace were 2−9.5 times higher in the plastic bottom enclosure (Fig. 1).

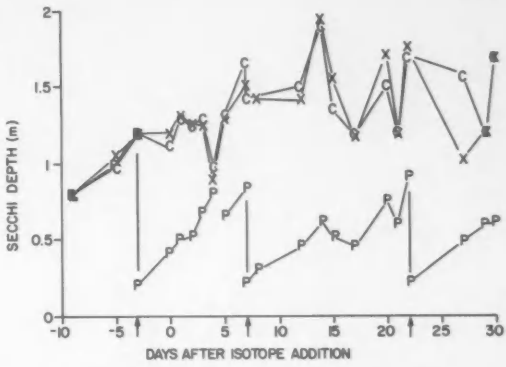

FIG. 2. Secchi depth readings in the controls (C and X) and the suspended sediment enclosure (P). Arrows on the horizontal axis indicate additions of sediment to the sediment addition enclosure.

Crayfish ^{75}Se bioaccumulation was also about 10 times higher in the plastic bottom enclosure (data not shown). In the absence of sediments in the plastic bottom enclosure, there was no detectable loss of ^{75}Se from the water during the experiment (Rudd et al. 1980b). The residence time of ^{75}Se in the water column of the control enclosure was 56 d (Rudd et al. 1980c).

1979 EXPERIMENT

Secchi depth in both control enclosures increased from 0.8 to 1.5 m between day −9 (i.e. 9 d before radioisotope addition) and day 8 (Fig. 2), and then remained at about 1.5 m (1.46 ± 0.24, ±SD, N = 26) for the remainder of the experiment. Secchi depth in the enclosure to which sediment was to be added tracked the controls until the first sediment addition on day −3, which reduced the Secchi depth to 0.2 m. Secchi depth increased until day 8 when a second addition of sediment returned the Secchi depth to 0.2 m. Another addition was made on day 22 after the Secchi depth had increased to 0.9 m. The average Secchi depth in the sediment addition enclosure after the first clay addition was 0.52 m (±0.20, N = 20). During the same time period, total suspended solids (TSS) in the control enclosures were 2.0 mg·L^{-1} (±0.0, N = 4). Total suspended solids were measured twice in the sediment addition enclosure when the Secchi depth was 0.3 m. On these occasions, TSS were 13 and 18 mg·L^{-1}, dry wt.

Phytoplankton primary productivity was generally similar in the control and the sediment addition enclosures throughout the experiment. However, immediately after sediment additions, the decreased light penetration reduced daily rates of primary productivity in the sediment addition enclosure by about a factor of 2 relative to the controls (~30 vs 60 mg C·m^{-2}·d^{-1}). This difference diminished with time because water transparency increased steadily after each sediment addition (Fig. 2). Such reductions in primary productivity are expected to have, at most, a small negative effect on rates of Hg bioaccumulation (Rudd and Turner 1983). This is

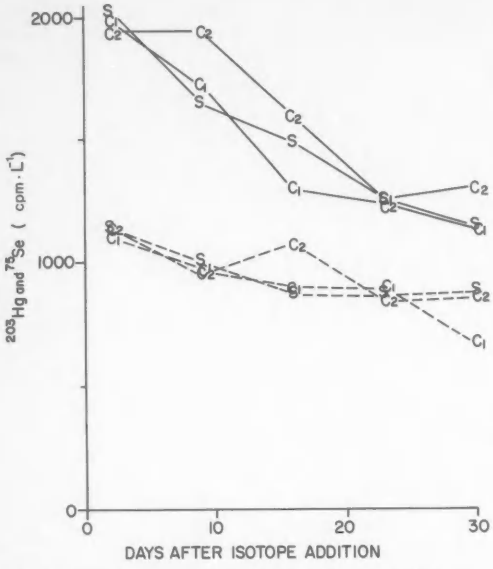

FIG. 3. Concentrations (cpm) of ^{203}Hg (solid lines) and ^{75}Se (broken lines) in water samples from the control enclosures (C_1 and C_2) and sediment addition enclosure (S) as a function of time. Radioisotope concentrations are sums of all Hg and Se fractions.

supported by our observations of zooplankton and pearl dace biomass. Zooplankton biomass was stable at approximately 0.15 mg dry wt · L^{-1} in all of the enclosures. Pearl dace mean weights were similar in all enclosures increasing by 12% in the control enclosures and by 10% in the sediment addition enclosure.

The addition of sediment had no effect on the rate of loss of either ^{203}Hg or ^{75}Se from the water column (Fig. 3). Concentrations of ^{203}Hg decreased by 45% during the course of the experiment and did not appear to have stabilized at the end of the experiment. During the experiment ^{75}Se concentration in water decreased by only about 10%, evidently reaching stable values (Fig. 3).

Although there was no overall effect of sediment addition on total concentration of ^{203}Hg in the water samples (Fig. 3), there was a significant increase in the percentage of ^{203}Hg on particulate material in the sediment addition enclosure with a corresponding decrease in the quantity of ^{203}Hg in the other fractions (Table 4). On the last day of the experiment, a fourth sediment addition was made to examine the short-term effects of newly added particulate material on the partitioning of the radioisotope. No discernable changes from the overall averages presented in Table 4 were observed in samples taken just before and after the sediment addition. The percentage of ^{203}Hg on suspended particulate material (43%, Table 4) is probably an underestimate because some very small clay-sized particles passed through the 0.45-μm membrane filters and were collected in a visible layer in the ion-exchange column. Conversely, one or more of the "dissolved" fractions will be overestimated because their radioisotopic concentrations were determined using filtered water.

Long-term partitioning of ^{75}Se was not affected by increased suspended sediment concentrations (Table 4). As in other experiments (Rudd et al. 1980b, 1980c; Turner and Rudd 1983), ^{75}Se in both the sediment addition and control enclosures was primarily associated with the particulate fraction (~55%) at the beginning of the experiment. This declined rapidly during the 1st wk of the experiment so that after day 9 about 55% of the ^{75}Se in all enclosures was associated with the organic fraction (activated charcoal). There may have been a short-term effect of sediment addition on the partitioning of ^{75}Se. Immediately after the fourth sediment addition on the last day of the experiment, the percentage of ^{75}Se in the organic fraction increased from 57 to 75%. This ^{75}Se was drawn entirely from the ionic fraction, which decreased from 32 to 14% of the total ^{75}Se concentration.

Biomass (grams per square centimetre) of periphyton on the polyethylene strips peaked on day 22 in all of the enclosures and was four times higher in the sediment addition enclosure than in the controls. On that day, ^{203}Hg and ^{75}Se concentrations per gram of periphyton were lower by 50 and 75%, respectively, in the suspended sediment enclosure than in the control enclosures.

The movement of both Hg and Se into the zooplankton, crayfish, and pearl dace was very similar in both control enclosures, usually varying by <10% between the control enclosures (data presented in Rudd and Turner 1983). This suggests that enclosure-to-enclosure variability was not a factor in interpreting the large differences in radioisotope concentrations of biota between the controls and sediment addition enclosures. Concentrations in zooplankton peaked early and then declined progressively appearing to follow the declining concentrations in water. Concentrations in pearl dace plateaued after 17 d of exposure. Both ^{203}Hg and ^{75}Se in crayfish increased continuously throughout the experiment.

The added sediment substantially reduced bioaccumulation of ^{203}Hg by the zooplankton (Fig. 4), crayfish (Fig. 4), clams (Table 5), and pearl dace (Fig. 5). Zooplankton concentrations were reduced to 20% of the controls, while ^{203}Hg concentrations of whole bodies of crayfish, clams, and pearl dace were reduced to 10−15% of the control enclosures. Movement of ^{203}Hg into pearl dace muscle was reduced even further. By the end of the experiment, ^{203}Hg concentrations in muscle of dace in the sediment addition enclosure were only 5% of those in the control enclosures (Fig. 5). At the end of this enclosure experiment, about 60% of the total ^{203}Hg in the muscle of the fish was $CH_3{}^{203}$Hg.

Bioaccumulation of ^{75}Se was also reduced by sediment addition, but to a smaller extent (Fig. 4 and 5; Table 5). In zooplankton, crayfish, and clams ^{75}Se concentrations were reduced to 50, 70, and 75% of the controls, respectively. In whole bodies of pearl dace, ^{75}Se concentration was reduced to about 60% of the controls. At the end of the experiment, ^{75}Se concentration in pearl dace muscle was reduced to 70% of the controls (Fig. 5).

Discussion

The water column and the sediments have been identified as sites of Hg methylation in aquatic ecosystems (Jacobs and

TABLE 4. Partitioning of ^{203}Hg and ^{75}Se in water samples taken from the 1979 control and sediment addition enclosures. Means (±SD) are reported for five samples taken at weekly intervals.

Enclosure	% particulate (>0.45 μm)	% charcoal (organic)	% ionic	% unassociated
		^{203}Hg		
Control 1	27.4 (±8.1)	24.9 (±8.3)	14.5 (±5.3)	33.1 (±15.3)
Control 2	26.5 (±4.7)	24.6 (±7.5)	13.4 (±4.8)	35.4 (±11.3)
Sediment addition	43.2 (±7.8)	16.1 (±5.0)	11.1 (±6.8)	29.7 (±9.5)
		^{75}Se		
Control 1	23.2 (±16.9)	51.1 (±12.3)	25.7 (±11.5)	—
Control 2	23.9 (±19.0)	55.0 (±14.4)	21.1 (±7.5)	—
Sediment addition	23.8 (±16.9)	54.7 (±16.7)	21.5 (±7.2)	—

Keeney 1974; Olson and Cooper 1974; Armstrong and Scott 1979; Huckabee et al. 1979; Furutani and Rudd 1980; Rudd et al. 1980a; Topping and Davies 1981). In the English–Wabigoon River system the water column and the organic-rich surface layer of the sediments are thought to be the major contributors of methyl mercury to fish (Rudd et al. 1983). The deeper organic-poor sediments, which underlie the surface organic floc, and which may contain high concentrations of Hg, are apparently not contributing substantially to the present methyl mercury levels in the biota (Rudd et al. 1983). Our enclosure experiments were designed to establish if the presence of organic-poor sediment in either the water column or on the bottom of the enclosures could reduce the bioaccumulation of methyl mercury. In the 1978 experiment, the organic-poor sediment was primarily on the bottom of the control enclosure with natural sediments — beneath the organic-rich surface sediment. This location of the organic-poor sediment was probably the cause of a twofold reduction in the residence time of ^{203}Hg in water as compared with the plastic bottom enclosure. This may have been partially responsible for the 10-fold reduction in Hg bioaccumulation by pearl dace and crayfish. Additionally, there was reduced ^{203}Hg bioavailability in the water column of the control enclosure with a natural sediment bottom because of resuspension of organic-poor sediment by wave action on the walls of the enclosures.

When organic-poor sediment from Wabigoon Lake (Table 2) was added directly to the water column in the 1979 experiment, there was no effect on residence time of ^{203}Hg in water (Fig. 3). Thus, in this experiment the large reduction observed in ^{203}Hg accumulation by the biota (Fig. 4 and 5), including a reduction to 5% of the controls in pearl dace muscle (Fig. 5), was likely a direct effect of suspended sediment. An overall interpretation of both experiments is that binding of ^{203}Hg and/or $CH_3{}^{203}Hg$ by the organic-poor sediment, either in the water column or on the bottom, was an effective inhibitor of Hg bioaccumulation.

An examination of the relative rates of loss of suspended sediment and ^{203}Hg from the water column of the 1979 sediment addition enclosure yielded information on the mechanism by which these organic-poor sediments reduced Hg bioaccumulation. Sedimentation of the larger particulate material resulted in increasing water clarity after each sediment addition (Fig. 2). However, this did not affect the water residence time of the labeled Hg (Fig. 3), although Hg bioaccumulation was drastically reduced. This suggests that the ^{203}Hg in the suspended sediment enclosure was quickly bound either to dissolved organics added as part of the wet sediment or to fine particulate material, which was permanently suspended or sedimenting very slowly. Because there was no detectable increase in dissolved or suspended organic carbon concentration in the suspended sediment enclosure as compared with the controls (data not shown), and because the amount of ^{203}Hg associated with the dissolved organic fraction was lower in the sediment addition enclosure (Table 4), it is likely that the labeled Hg was bound by the clay mineral fraction of the suspended sediments. Mercury is known to bind to clay particles both by ion-exchange reactions and by coprecipitation with organics or inorganics (Fe, Mn) onto the surfaces of clay-sized particles (Jonasson 1970; Lockwood and Chen 1974), and in fact, an increase in percentage of Hg on particulate material was observed in the sediment addition enclosure (Table 4). This observation was in agreement with Armstrong and Hamilton (1973) who found that Hg was associated primarily with the clay-sized fraction of Clay Lake sediments. In addition, Lindberg and Harriss (1977) concluded that Fe and not organics was responsible for the scavenging of Hg after resuspension of sediments in laboratory experiments. Also, Jackson et al. (1984) concluded that particulate-bound Hg was not readily available for bioaccumulation. All of these observations indicate that in our enclosure experiments the binding of Hg to clay-sized particles, in both the water column and surface sediments, reduced the bioavailability of Hg.

This Hg binding process was probably operative in Clay Lake, since suspended sediment concentrations in the lake and in the 1979 enclosures were quite similar. Concentrations of suspended sediment, as measured by Secchi depth, averaged 0.5 m (±0.2, $N = 20$) (Fig. 2) in the sediment addition enclosure and 0.6 m (Table 3) in the eastern basin of Clay Lake. Suspended sediment concentrations in the western basin of Clay Lake, which had greater water clarity, were more closely approximated by the 1979 control enclosures. The western basin Secchi depth averaged 1.27 m (Table 3) as compared with 1.46 m (0.24, $N = 26$) (Fig. 2) in the control enclosures.

To determine if the same type of binding mechanism observed in the enclosures was operative in Clay Lake, an

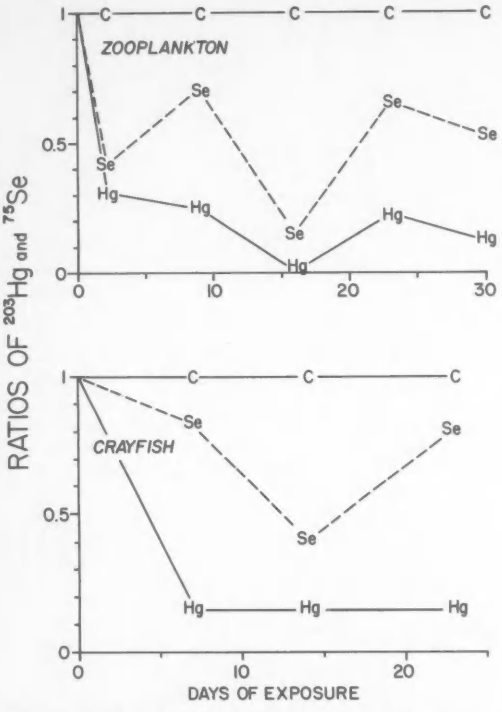

FIG. 4. Inhibition of ^{203}Hg and ^{75}Se bioaccumulation by zooplankton and crayfish by addition of organic-poor sediment to an enclosure. The values presented are ratios in which ^{203}Hg and ^{75}Se concentrations in biota of the sediment addition enclosure are divided by the corresponding concentrations of the combined control enclosures (C).

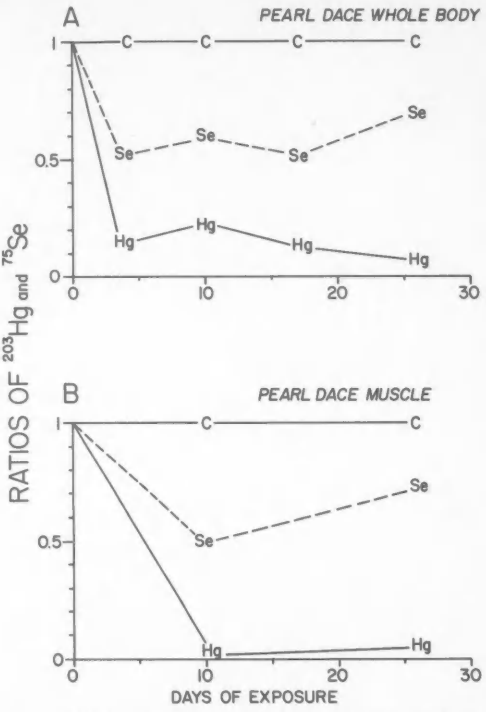

FIG. 5. Inhibition of ^{203}Hg and ^{75}Se bioaccumulation in pearl dace whole body and muscle samples by addition of inorganic-rich sediment. Values presented are ratios in which ^{203}Hg and ^{75}Se concentrations in biota of the sediment addition enclosure are divided by the corresponding concentrations of the combined control enclosures (C).

TABLE 5. Accumulation of ^{203}Hg and ^{75}Se in the foot muscle of clams in the presence and absence of added sediment. The lognormal mean of 5 clams is reported for the sediment addition enclosure and of 10 clams for the combined controls. One standard deviation is obtained by multiplying or dividing the lognormal mean by the factor given in parentheses.

Enclosure	^{203}Hg (cpm·g^{-1} $\overset{\times}{\div}$ SD)	^{203}Hg (% of controls)	^{75}Se (cpm·g^{-1} $\overset{\times}{\div}$ SD)	^{75}Se (% of controls)
Combined controls	265 ($\overset{\times}{\div}$1.28)	100	190 ($\overset{\times}{\div}$1.48)	100
Sediment addition	33 ($\overset{\times}{\div}$1.63)	13	143 ($\overset{\times}{\div}$1.73)	75

experiment examining Hg bioaccumulation by caged fish was carried out at the inflow and outflow of the lake where suspended sediment concentrations were quite similar to concentrations in the eastern and western basins (Table 3). During the 8-wk experiment suspended sediment concentrations and concentrations of total Hg, which was predominantly inorganic Hg, were higher at the inflow of the lake than at the outflow (Fig. 6; Table 3). Even though the Hg concentrations at the inflow were higher, both the finescale dace and pearl dace accumulated mercury more slowly at the inflow than at the outflow where the suspended sediment concentrations were lower (Fig. 7; Table 3). This indicates that the Hg at the outflow was more available for methylation and bioaccumulation. The higher concentrations of methyl mercury at the outflow (Fig. 6) and the much higher ratio of methyl mercury to total Hg at the outflow (Table 3) also support this conclusion. The results of this experiment are in agreement with the observations of Parks et al. (1980) and Jackson and Woychuk

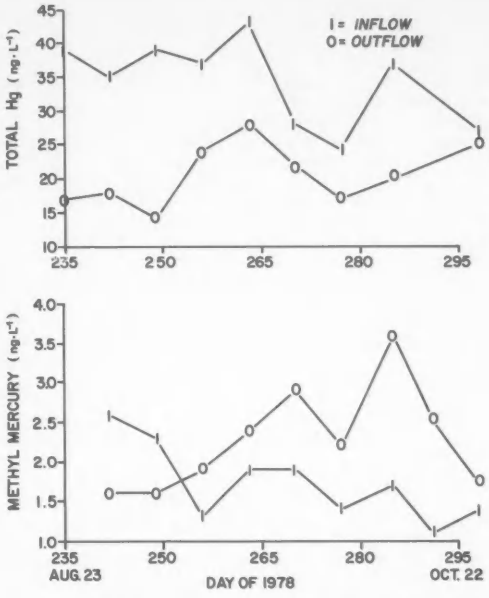

FIG. 6. Concentrations of total Hg and methyl mercury at the inflow and outflow of Clay Lake during the late summer and fall of 1978.

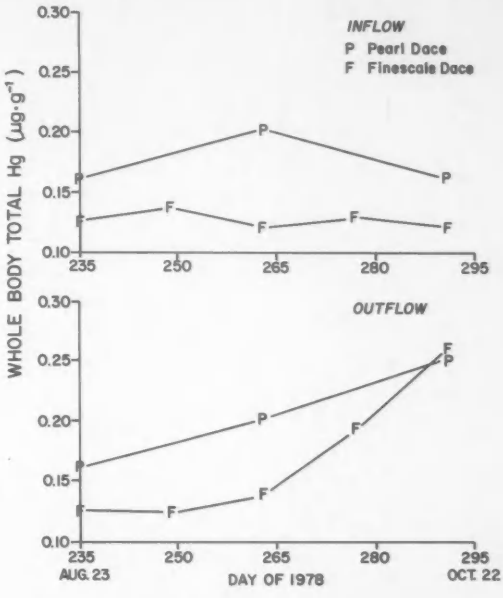

FIG. 7. Total Hg concentrations in finescale and pearl dace caged at the inflow and outflow of Clay Lake during the late summer and fall of 1978.

(1980) who found a progressive increase in the ratio of methyl mercury to total Hg in the water of this river–lake system with increasing distance downstream from Dryden. As we observed (Table 3), the increasing ratio of methyl mercury to total Hg also corresponded to decreasing downstream suspended sediment load.

INHIBITION OF SELENIUM BIOACCUMULATION

We were interested in the extent to which resuspension of sediment would alter Se bioaccumulation because trace Se addition was being considered as a Hg ameliorating procedure (Rudd et al. 1983; Turner and Rudd 1983). This type of information is also useful in assessing the possible toxicity or ameliorative effects of Se added to waterbodies from industrial sources or by coal combustion (Klein et al. 1975).

In the 1979 experiment, addition of organic-poor sediment reduced the rate of Se bioaccumulation to 50–75% of the controls in all members of the food web tested (Fig. 4 and 5; Table 5). A similar effect was observed in a companion experiment in which an increased rate of primary productivity decreased Se concentration in biota (Rudd and Turner 1983). In that case, Se appeared to be diluted by the increased rate of growth of the organisms. In the 1979 sediment addition experiment, the reduction was a direct effect of the organic-poor sediment, since growth rates of fish and phytoplankton were not significantly different from those in the controls.

In the 1978 experiment, the presence of natural sediments was correlated with a much larger (10-fold) reduction in Se bioaccumulation by pearl dace (Fig. 1) and crayfish (data not shown). At trace concentrations (<0.2 μg $Se \cdot L^{-1}$), Se is known to be predominantly associated with organic material (Table 2; Rudd et al. 1980b, 1980c; Rudd and Turner 1983; Turner and Rudd 1983). During the 1978 experiment, organic-rich material on the surface of the natural sediments may have bound the ^{75}Se reducing its bioavailability. This could have occurred on the bottom of the enclosure or after resuspension of the organic-rich material by wave action on the enclosure walls. During the 1979 experiment, predominantly organic-poor sediments were added to the sediment addition enclosure. These observations suggest that presence of organic-rich sediment may have a larger inhibitory effect on Se bioaccumulation than organic-poor sediments.

The present Hg:Se atomic ratios of northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) in Clay Lake are about 10:1 (Rudd et al. 1980b). Our observations that organic-poor sediment addition reduced muscle Hg concentration to 5% of the controls, and Se concentration to only 70%, suggest that the Hg:Se ratio of fish in the English–Wabigoon system could be reduced (improved) by addition of organic-poor sediments to the water column and surface sediments of this Hg-polluted river system.

USE OF SEDIMENT SUSPENSION AS A MERCURY AMELIORATING PROCEDURE

Continuous or periodic resuspension of sediment by small dredges followed by downstream deposition might be a

feasible amelioration procedure for the Hg-polluted English–Wabigoon River system in which a large quantity of Hg is being continuously transported downstream (Rudd et al. 1983). Sediment resuspension followed by downstream deposition would attack the problem in both the water column and the surface sediments — the two most important contributors of Hg to the biota (Rudd et al. 1983). In the water column and surface sediments, resuspended sediments would bind Hg and reduce its bioavailability. Further, at the sediment surface, increased deposition of clean, organic-poor material would dilute concentrations of both Hg and organic carbon, the latter being a source of bacterial food. Both of these factors can control rates of microbial Hg methylation in Clay Lake surface sediments (Furutani and Rudd 1980; Rudd et al. 1983). In support of our conclusions, Parks et al. (1984) found that methyl mercury concentrations in fish are correlated with local water and surface sediment Hg concentrations throughout the river system.

The approach described above might prove useful for other heavy metal contamination problems. For example, the bioaccumulation of ^{65}Zn by crayfish in the 1978 study was eightfold higher in the enclosure with a sealed plastic bottom than in the enclosure with natural lake sediments. Apparently, binding of ^{65}Zn to resuspended sediments, or to particles at the sediment–water interface of the enclosure with natural sediments, effectively inhibited zinc bioaccumulation.

Before sediment resuspension is used as a remedial measure for Hg pollution, several potential problems should be considered. First, although pearl dace growth was not significantly reduced in the 1979 experiment, the effects of increasing suspended sediments on fish productivity should be further investigated. Second, a safe ratio of clean to contaminated sediments should be determined. The 1978 experiment using Hg-polluted Clay Lake sediments and the laboratory experiments of Lindberg and Harriss (1977) suggest that resuspension and downstream deposition of some contaminated sediments might not be a problem. Third, the possibility that Hg attached to suspended particles could be released downstream in response to changes in pH, ionic strength, or chloride concentrations should be considered. For example, Hg adsorbed to kaolinite, bentonite, illite, and iron oxide at neutral pH is greater than 50% desorbed between pH 4.5 and 5.0 and is at least 75% desorbed at pH 4.0 (Andersson 1979). While such downstream changes are not expected in the English–Wabigoon River system, this possibility should be fully investigated for other aquatic systems.

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The English–Wabigoon River System: III. Selenium in Lake Enclosures: Its Geochemistry, Bioaccumulation, and Ability to Reduce Mercury Bioaccumulation

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Aquatic biota have been shown previously to bioaccumulate selenium rapidly with a concomitant reduction in accumulation of mercury when exposed to 100 μg Se/L. Using radioisotope techniques this experiment extends these observations by examining the effects of single additions of sodium selenite at 1, 10, and 100 μg Se/L to experimental ecosystems (130 m^3) located in mercury-contaminated Clay Lake, northwestern Ontario (50°03′N, 90°30′W). The major sink for added selenium and mercury was the sediment. Movement of ^{75}Se into the sediments in control enclosures (<0.2 μg Se/L) appeared to stabilize within 6 wk; however, movement did not stabilize in the selenium enclosures where selenium remaining in water was largely ionic. At first, selenium accumulated rapidly in net plankton but then declined in correspondence with water concentrations. In *Anadonta* sp. and *Orconectes virilis*, selenium increased with time and in proportion to ambient selenium. Stable concentrations of selenium were not achieved in fish after 2 mo, but levels always varied according to the following sequence: *Semotilus margarita* < *Perca flavescens* < *Catostomus commersoni*, ranging from 0.2 to 0.4 μg Se/g (wet wt) at 1 μg Se/L. Accumulation of mercury was reduced in several biota in a concentration-dependent manner, with greatest reductions observed at 100 μg Se/L. These reductions appeared to depend on the position of the organism in the food web. In fish, the reduction was proportional to the amount of selenium accumulated. If not present in excessive amounts, selenium might be of benefit in the treatment of aquatic ecosystems with either atmospheric or point-source mercury pollution problems.

TURNER, M. A., AND J. W. M. RUDD. 1983. The English–Wabigoon River system: III. Selenium in lake enclosures: its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Can. J. Fish. Aquat. Sci. 40: 2228–2240.

Nous avons déjà démontré que des biocénoses aquatiques bioaccumulent le sélénium rapidement en même temps que diminue l'accumulation du mercure en présence de 100 μg Se/L. L'expérience décrite dans l'article qui suit, menée à l'aide de radioisotopes, élargit la portée de ces observations en étudiant les effets d'additions uniques de sélénite de sodium à des concentrations de 1, 10 et 100 μg Se/L à des écosystèmes expérimentaux (130 m^3) dans le lac Clay, dans le nord-ouest de l'Ontario (50°03′N, 90°30′W), contaminé par le mercure. Le sédiment est le réservoir principal du sélénium et du mercure ajoutés. Le mouvement de ^{75}Se dans les sédiments d'enceintes témoins (<0,2 μg Se/L) semble se stabiliser en moins de 6 sem; cependant, le mouvement ne se stabilise pas dans les enceintes contenant du sélénium, dans lesquelles ce qui reste de ce dernier dans l'eau est en grande partie ionique. Au début, le sélénium s'accumule rapidement dans le plancton tamisé, mais diminue ensuite en même temps que diminuent les concentrations dans l'eau. Chez *Anodonta* sp. et *Orconectes virilis*, le sélénium augmente en fonction du temps et en proportion de sa concentration ambiante. Les concentrations de sélénium dans les poissons ne sont pas encore stabilisées après 2 mois, mais les niveaux varient dans l'ordre suivant : *Semotilus margarita* < *Perca flavescens* < *Catostomus commersoni* et sont compris entre 0,2 et 0,4 μg Se/g (poids humide) à 1 μg Se/L. Dans plusieurs biocénoses, l'accumulation du mercure diminue en fonction de la concentration, les plus fortes diminutions se produisant à 100 μg Se/L. Ces diminutions semblent dépendre de la position de l'organisme dans le réseau

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alimentaire. Chez les poissons, la diminution est proportionnelle à la quantité de sélénium accumulé. Si cette dernière n'est pas excessive, le sélénium peut être favorable dans le traitement des écosystèmes aquatiques où il y a contamination par le mercure soit atmosphérique, soit de sources ponctuelles.

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In the past decade point-source Hg pollution has been recognized as a severe environmental problem (National Academy of Sciences 1978; National Research Council 1979). Mercury present in emissions from fossil fuel combustion (Klein et al. 1975) can also be transported into the aquasphere (Gutenmann et al. 1976; Huckabee and Blaylock 1974) and be of public concern. Furthermore, acidification of rainfall may alter the chemical form of Hg to aggravate the problem (National Academy of Sciences 1981). A combination of these processes may explain high Hg concentrations found in fish in lakes undergoing acidification (Jernelov et al. 1976; Brouzes et al. 1977).

Selenium is also found in emissions from burning of fossil fuels (Hashimoto et al. 1970). It has been estimated that anthropogenic emissions of Se into the atmosphere in the United States are about 10 times those of Hg on an atomic basis (Klein et al. 1975). Although the distance Se can be transported atmospherically is not well known, Se can be scavenged by rainfall in a manner similar to particulate trace metals (Hicks 1979). Consequently, Se is found at elevated concentrations in soils and waters near the source (Gutenmann et al. 1976; Fingleton and Robbins 1980; Crocket and Kabir 1981).

Selenium is an essential trace element with chemical properties similar to sulfur (Frost and Lish 1975; Vokal-Borek 1979). However, it is toxic in excess and is not permitted in drinking water in Canada at concentrations above 10 μg Se/L (National Health and Welfare 1978; DeMayo et al. 1979). Selenium can reduce the toxicity of several heavy metals (Ag^+, Cd^{2+}, Hg^{2+}, and As, reviewed by Frost and Lish 1975 and Vokal-Borek 1979) and, relevantly to our studies, can reduce the severity of inorganic and methyl mercury poisoning in consumers of contaminated food (Chang et al. 1977; National Academy of Sciences 1978). Selenium can also reduce accumulation of Hg in pearl dace (*Semotilus margarita*) and crayfish (*Orconectes virilis*); moreover, these animals accumulate Se efficiently at both trace (<0.2 μg Se/L) and increased (~100 μg Se/L) concentrations (Rudd et al. 1980b). Therefore, it is possible that atmospherically transported Se, or small amounts of Se added to a polluted waterway, might alleviate Hg pollution problems both by reducing concentrations of Hg in fish and by rendering the remaining Hg less toxic to fish consumers. Moreover, for Se to exert its Hg-detoxifying effect, Hg concentrations do not have to be decreased in either the food or consumer, and in some cases, Hg concentrations can even be higher (Chang et al. 1977; Friedman et al. 1978).

Clay Lake (50°03′N, 90°30′W), which is part of the Wabigoon River system, was the site of the present research (Rudd et al. 1983). For the past two decades, it has been polluted severely by Hg discharged from a pulp and paper mill at Dryden, northwestern Ontario (Armstrong and Hamilton 1973; Parks et al. 1980). In this experiment we have studied the uptake of Se and Hg (using radioisotopes) into several fish species, zoobenthos, and plankton. This was done in the field in specially constructed enclosures to which Se was added and from which biota, sediment, and water were sampled for up to 60 d. The results are considered from the point of view of Se as a Hg ameliorating agent.

Methods

The enclosure methodology used in this experiment is similar to that described in Rudd et al. (1980a). Over the period of 1 d, polyethylene-walled experimental cylinders (surface and sediment area ~79 m^2, wall area ~52 m^2) were sealed into the clay sediments of a sheltered bay in the eastern basin of Clay Lake enclosing ~130 m^3 of lake water. The general experimental design consisted of five enclosures: two controls and one enclosure each at initial concentrations of 1, 10, and 100 μg Se/L. Note that although concentrations of Se in water declined subsequently with time in the enclosures, to prevent confusion, enclosures will be identified by their initial Se concentrations.

Chemical Analyses

Samples for water chemistry were taken at 0, 3, and 6 wk. No consistent differences were observed among the enclosures in suspended or dissolved carbon, nitrogen, and phosphorus species as analyzed according to the methods of Stainton et al. (1977). Total Hg and methyl mercury concentrations (~25 and ~1.5 ng Hg/L, respectively) were measured (Furutani and Rudd 1980) and were found to be similar among the enclosures. Concentrations of stable isotopes of Se in Clay Lake water were below the analytical detection limit of 0.2 μg total Se/L.

Radiochemical Analyses

Each enclosure received separate additions of 5 mCi of $^{203}Hg(NO_3)_2$ and 3 mCi of $H_2{}^{75}SeO_3$ (1 Ci = 37 GBq) (New England Nuclear Corp.) on July 15, 1979. Approximately 15 ng Hg/L, as $HgCl_2$, was added as carrier with the Hg upon final dilution in the enclosures. Because ^{75}Se was mixed with the Na_2SeO_3 and subsequently added to the 1, 10, and 100 μg Se/L enclosures, its concentration in biota of these enclosures can be used to calculate rates of Se accumulation. In contrast, ^{203}Hg was used in a comparative approach, not as a radiotracer, permitting detection of treatment effects by contrasting accumulations of ^{203}Hg in biota of the control and Se-treated enclosures. The radioisotope rationale is presented in detail in

Rudd and Turner (1983). Mercury and Se radioisotopes were assayed using gamma spectroscopy techniques as described in Rudd and Turner (1983).

The relationship between methyl and total Hg in muscle samples was assayed using a combination of gamma and scintillation counting techniques. Samples were originally counted using gamma spectroscopy and then extracted with toluene for radiolabeled methyl mercury according to the technique of Uthe et al. (1972) except that the last stage required to purify the sample prior to injection into a gas chromatograph was not performed. The percent recovery of methyl ^{203}Hg into the toluene phase was assumed to be 100%. Eight millilitres of the toluene phase was recovered from the extraction. This toluene was then added to 12 mL of Phase Combining System cocktail (Amersham/Searle) and counted using a Packard liquid scintillation counter employing the channels ratio technique. External standards were used to compare techniques.

Geochemical Samples

Water and haptobenthic[1] samples were taken, processed, and analyzed as described elsewhere (Rudd and Turner 1983). Duplicate cores, sectioned at 1-cm intervals to a maximum depth of 5 cm, were analyzed on each sampling occasion.

Biological Samples

Weekly net plankton samples were taken using a modified Pennak tube (Salki 1981) in which 60 L of water was passed through a 73-μm mesh net. The concentrated samples (approximately 100 mL) were preserved in the field by successive additions of 5 mL of ethanol and formalin. To permit radioassay, samples were filtered through preweighed Whatman GF/C filters and dried at 60°C. Samples consisted of similar amounts of algae and zooplankton (A. Salki, Freshwater Institute, Winnipeg, Man., personal communication). Most biological samples collected for radioassay were frozen immediately upon collection using dry ice and maintained in a frozen condition until radioassay. Adult crayfish and clams (*Anadonta* sp.) were handled as described in Rudd and Turner (1983).

Snails (~2 g) were collected from Clay Lake and two dozen added to each enclosure 1 wk after radioisotope addition. Whole bodies, excluding the shells, were assayed for radioisotope concentration after 1 and 2 wk of exposure.

One day after radioisotope addition, 70 adult pearl dace (5.0 ± 0.7 g, mean ± SD of a random sample of 38), obtained from a nearby uncontaminated lake, were added to each of the enclosures. To obtain estimates of whole body radioisotope concentration, at least six pearl dace were sampled weekly using baited minnow traps tended hourly. Every 2nd wk, skeletal muscle samples were obtained from four dace selected randomly from each collection.

In addition to young-of-the-year yellow perch (*Perca flavescens*) trapped during emplacement of the enclosures, 13 perch obtained from the bay were added to each enclosure 3 d after radioisotope addition. Three perch were sampled every 2nd wk for whole body analysis.

Several young-of-the-year yellow perch and juvenile white suckers (*Catostomus commersoni*) were trapped during installation of the enclosures. Most were removed by trapping prior to radioisotope addition. From subsequent sampling and by addition of rotenone (about 8 mg/L) to the enclosures at the end of the experiment, the densities of fish species remaining were determined. Consequently, with identical experimental fish additions of dace (48 kg/ha) and perch (1.5 kg/ha) made to each enclosure, initial fish densities were similar among the enclosures, ranging from 51.2 to 65.9 kg/ha.

Results

Mercury and Selenium Geochemistry

With the exception of the 100 μg Se/L enclosure, disappearance of ^{203}Hg from water was similar among all enclosures (Fig. 1A). This was not the case for ^{75}Se (Fig. 1B). Loss of ^{203}Hg from the water column followed first-order kinetics ($0.966 \leq r^2 \leq 0.991$) (Table 1). Calculated half-times ranged from 20.3 to 22.7 d; but at 100 μg Se/L, a half-time of 14.0 ± 0.6 d (SD) was observed (Table 1). This was confirmed by observation of the complementary increases in ^{203}Hg concentration in the sediments (Fig. 1C).

Elevated Se concentrations did not detectably alter partitioning of ^{203}Hg among particulate, ionic, and undefined[2] fractions (Table 2). However, the overall mean proportion of ^{203}Hg in the activated charcoal fraction appears to have been lowered from 30 ± 0% (SE) in the combined controls to 23 ± 1% (SE) at elevated Se concentrations.

The residence time of Se in water was unaffected by increasing the initial concentration from 1 to 100 μg Se/L (Table 3), with Se concentrations in water continuing to decrease after 6 wk (Fig. 1B). In contrast, in the controls, with background concentrations of Se (<0.2 μg Se/L), ^{75}Se concentration in water appeared to stabilize at one-half the initial value after 4 wk (Fig. 1B). This apparent difference in pattern of movement agreed with sediment concentrations (Fig. 1D). Observations of longer duration, or increased sediment to water ratio, are required to confirm this difference.

There was a substantial shift of ^{75}Se from the charcoal to ionic fraction with increased concentration of Se, especially noticeable in a comparison between control and Se enclosures (Table 2), but also significantly different between the 1 and 100 μg Se/L enclosures ($P < 0.05$, paired t-test). Earlier observations indicate that this ionic Se was likely to be predominantly anionic (Rudd et al. 1980a). In the control enclosures, the percentage on particles decreased rapidly with time, declining from 49% on day 2 to 13% on day 9 (not shown). In contrast, the particulate fraction in the Se enclosures was always observed to be very low, usually about 2% (Table 2).

[1]"Haptobenthos" is the community associated with solid substrates (Hutchinson 1975). The term haptobenthos is preferred to "periphyton" because it emphasizes that the community consists of several classes of organisms and can include nonliving material, whereas the latter term is frequently used to describe only benthic algae.

[2]The undefined fraction represents dissolved ^{203}Hg (<0.45 μm) that was not associated with any of the other fractions but could be precipitated as HgS after a sulfuric acid–persulfate digestion.

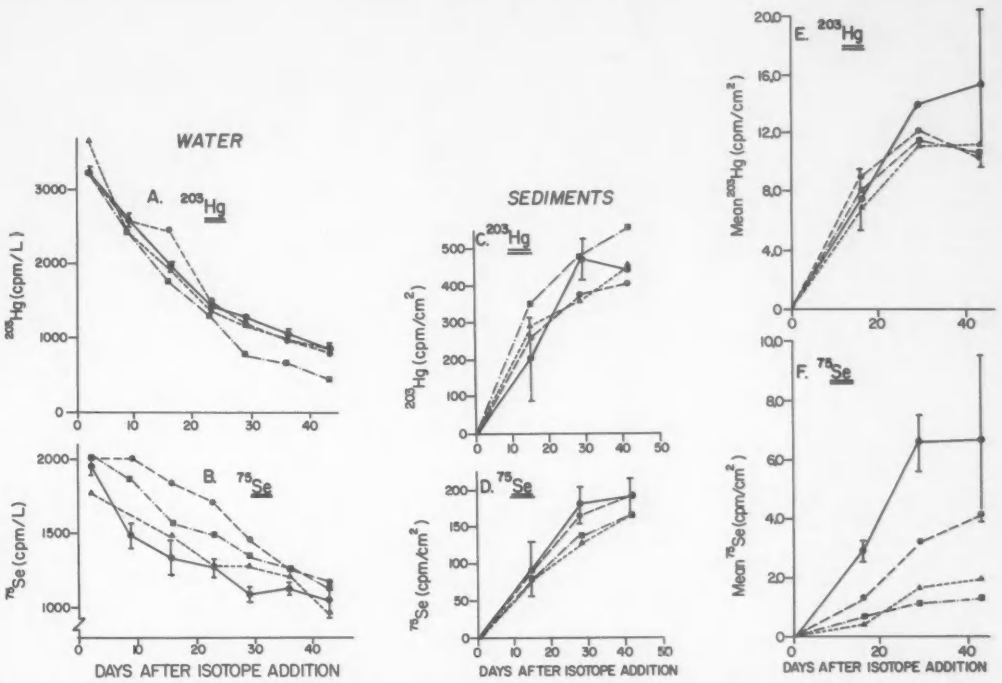


FIG. 1. Movement of ^{203}Hg and ^{75}Se among several compartments in the controls (●) and 1 (*), 10 (▲), and 100 (■) μg Se/L enclosures showing loss of ^{203}Hg (A) and ^{75}Se (B) from the water column, accumulation of ^{203}Hg (C) and ^{75}Se (D) in sediments, and accumulation of ^{203}Hg (E) and ^{75}Se (F) in haptobenthos. For each sampling date the mean of the controls is presented with a vertical line connecting the individual values. Given the known specific activity of added ^{75}Se:Se in the enclosures, actual Se concentrations in water could be calculated (Fig. 1B). Axes appropriate to each Se enclosure are presented on the right side of the figure. Maximum ^{75}Se concentrations in sediment (Fig. 1D) were observed in the upper 1 cm and correspond to concentrations of 0.051, 0.60, and 4.4 μg Se/g wet wt in the 1, 10, and 100 μg Se/L enclosures, respectively. Similarly, maximum concentrations found in the haptobenthos (Fig. 1F) of the enclosures were 16, 114, and 1460 μg Se/g dry wt.

TABLE 1. Movement of ^{203}Hg out of the water in the enclosures using the first-order kinetics equation $C_t = C_0 \times e^{(-\ln 2 \times \Delta t/t_{0.5})}$. C_t is the radioisotope concentration at time Δt (days after addition), C_0 is the initial concentration, and the rate of change in concentration is expressed as $\log_e/t_{0.5}$ with $t_{0.5}$ equal to the half-time of the isotope in the water column (Hesslein et al. 1980). $N = 7$ for each treatment condition.

		Control		Se		
Parameter	Units	1	2	1	10	100
r^2		0.987	0.989	0.966	0.968	0.991
C_0(±SD)	cpm/L	3429(±22)	3328(±16)	3569(±32)	3498(±40)	3641(±24)
$t_{0.5}$(±SD)	d	20.8(±1.2)	22.7(±1.1)	20.7(±1.7)	20.3(±1.9)	14.0(±0.6)

Haptobenthic areal concentrations of ^{203}Hg (i.e. ^{203}Hg per square centimetre) increased until the 4th wk of exposure (Fig. 1E). After 2 wk, rate of bioaccumulation of ^{203}Hg per unit mass (i.e. ^{203}Hg per gram) decreased with time (not shown) paralleling water concentrations. This probably reflects the interaction of increasing haptobenthic biomass with decreasing concentration of radioisotope in water (Fig. 1A).

Elevated Se levels did not influence haptobenthic accumulation of ^{203}Hg (Fig. 1E) unlike the earlier experiment (Rudd et al. 1980a). However, in that study the response of an established community was observed, whereas the more recent observation is that of a newly developing community.

Areal accumulation of ^{75}Se in the wall haptobenthos was inversely proportional to stable Se concentration (Fig. 1F). In the 10 and 100 μg Se/L enclosures, ^{75}Se concentration per unit dry weight of haptobenthos (not shown) declined

TABLE 2. Partitioning of ^{203}Hg and ^{75}Se in water into particulate, charcoal, ionic, and undefined (^{203}Hg only) fractions. Percentage means of the time series (±SD, $N = 7$) are presented ($N = 6$ for ^{75}Se in the 10 μg Se/L enclosure).

		Control (<0.2 μg Se/L)		Se (μg Se/L)		
Isotope	Fraction	1	2	1	10	100
^{203}Hg	Particulate (>0.45 μm)	29.5 (±15.1)	19.0 (±2.2)	19.4 (±4.3)	20.4 (±2.9)	26.1 (±8.3)
	Activated charcoal	29.6 (±6.5)	29.9 (±9.0)	22.8 (±7.3)	24.2 (±3.3)	22.4 (±4.5)
	Ionic	13.4 (±3.8)	17.5 (±3.1)	18.8 (±7.8)	15.6 (±6.3)	18.8 (±4.4)
	Undefined	27.6 (±11.6)	33.6 (±9.5)	39.0 (±7.9)	39.8 (±10.2)	32.8 (±8.9)
^{75}Se	Particulate (>0.45 μm)	15.8 (±16.2)	14.4 (±15.9)	2.1 (±0.2)	1.5 (±0.5)	2.2 (±3.2)
	Activated charcoal	59.4 (±12.2)	62.3 (±14.8)	27.3 (±7.1)	23.8 (±2.9)	18.8 (±3.4)
	Ionic	22.1 (±5.0)	23.4 (±8.0)	70.1 (±7.2)	74.7 (±3.1)	79.0 (±4.3)

TABLE 3. Movement of ^{75}Se out of the water in enclosures using $(C_t - C_e) = (C_0 - C_e) \times e^{(-\ln 2 \times \Delta t / t_{0.5})}$ for the controls and the special case of $C_e = 0$, or $C_t = C_0 \times e^{(-\ln 2 \times \Delta t / t_{0.5})}$, for the situation of added Se.[a] C_t, C_0, and $t_{0.5}$ are as described in Table 1. C_e is the equilibrium concentration of ^{75}Se in water (R. H. Hesslein, Freshwater Institute, personal communication). N usually equals 7. $N = 6$ for the 10 μg Se/L enclosure.

		Control		Selenium		
Parameter	Units	1	2	1	10	100
r^2		0.974	0.953	0.946	0.944	0.980
C_0(±SD)	cpm/L	2180(±45)	2093(±55)	2231(±12)	1845(±12)	2080(±7)
C_e(±SD)	cpm/L	1089(±31)	984(±33)	0	0	0
$t_{0.5}$(±SD)	d	8.2(±0.1)	7.6(±1.0)	47(±5)	50(±5)	48(±3)

[a]The simpler version of the model, with $C_e = 0$, provided poorer fits to the control enclosure observations ($r^2 \sim 0.88$) than did the model incorporating nonzero equilibrium values ($r^2 > 0.95$). With equilibrium values differing between models, residence times are not comparable directly between models and therefore between control and Se conditions.

after 2 wk of exposure. Selenium concentration continued to increase throughout the experiment in the 1 μg Se/L enclosure. Maximum observed Se concentrations in haptobenthos were 16, 114, and 1460 μg/g (dry wt) in the 1, 10, and 100 μg Se/L enclosures, respectively.

For these experiments, initiated without established wall haptobenthos, the major geochemical sink for both radioisotopes was the sediment. One-half and two-thirds of the ^{75}Se and ^{203}Hg, respectively, was present in the sediments after 6 wk, while less than 2% of either radioisotope was found in the haptobenthos.

SELENIUM BIOACCUMULATION AND TOXICITY

Selenium-75 concentrations of net plankton in both controls (Fig. 2A), and to a lesser extent at 1 μg Se/L (Fig. 2B), rose rapidly and then declined, approximately following water concentrations (Fig. 1B). Maximum Se concentrations were attained progressively later at 10 and 100 μg Se/L (Fig. 2B). Selenium-75 in crayfish (Fig. 2A and 2B), snails, and clams (not shown) increased continuously over the 40-d exposure, with the exception of 100 μg Se/L enclosure-treated crayfish in which Se concentrations stabilized with time (Fig. 2B).

Concentration of Se within all invertebrates studied was proportional to ambient Se concentration (e.g. Fig. 2B). At 100 μg Se/L, all invertebrates appeared to achieve stable concentrations: crayfish whole bodies (0.8 μg Se/g wet wt), snail whole bodies (4.0 μg Se/g), and clam foot muscle (1.2 μg Se/g). In the 1 and 10 μg Se/L enclosures, Se concentrations in crayfish continued to increase rapidly after 6 wk (Fig. 2B).

Whole body Se concentrations in fish increased throughout the experiment and in proportion to the ambient Se concentration (Fig. 3). Concentrations increased consistently according to the following species sequence: adult pearl dace < young-of-the-year yellow perch < young white sucker (Fig. 3).

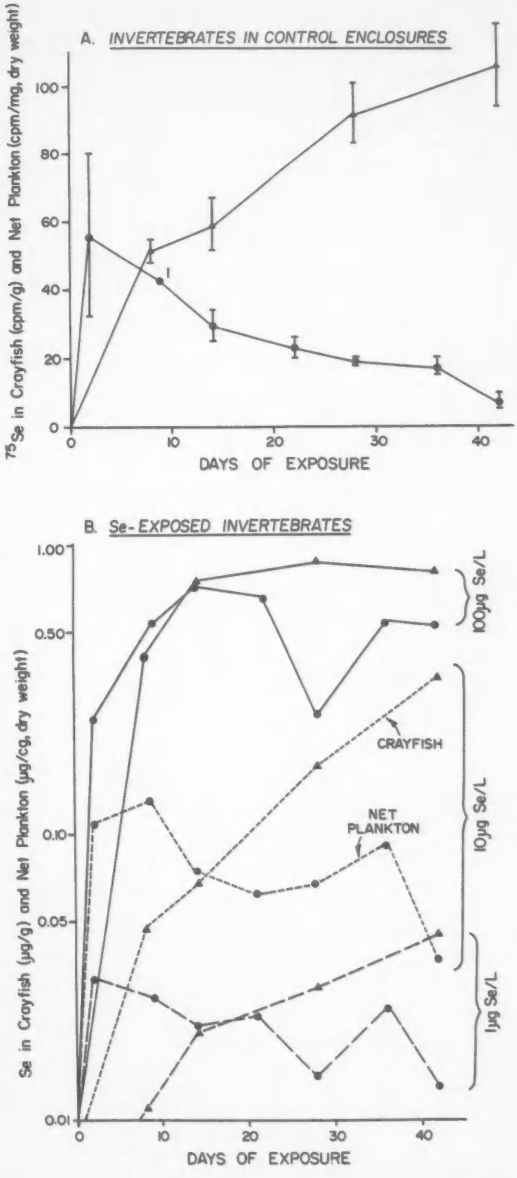


FIG. 2. (A) Accumulation of ^{75}Se in net plankton (●) and crayfish whole bodies (▲) in the control enclosures. Means and individual values are presented as in Fig. 1. Only one control (superscript 1) was sampled for net plankton on the second date. (B) Selenium bioaccumulation by invertebrates in the elevated Se enclosures was calculated based on known specific activities of added Se: 1 (– –), 10 (---), and 100 (—) µg Se/L.

The proportion of Se in skeletal muscle, relative to the whole bodies of pearl dace, increased both with the ambient level of Se and with the duration of exposure. The overall ratio of concentrations of muscle Se to whole-body Se in dace of the two controls was 33 ± 0% (SE, with each overall control mean comprising means of three or four observation periods). Corresponding percentages at 1, 10, and 100 µg Se/L were 38, 55, and 70%, respectively. Characteristically, the proportion of Se in skeletal muscle increased with time, with the proportion increasing from wk 2 to 8 by 36 ± 3% (SD, $N = 5$) in pearl dace of the control and Se enclosures.

MERCURY BIOACCUMULATION

The relationship between methyl and total ^{203}Hg in skeletal muscle of pearl dace is a sigmoidal function of time (Fig. 4A). Initially, the proportion of methyl to total ^{203}Hg was low, but between 4 and 6 wk of exposure, there was a rapid increase. After 8 wk, the proportion of methyl to total ^{203}Hg was 75% (±12%, SD, $N = 3$), 85% (±3%), and 101% (±5%) in pearl dace, yellow perch, and white suckers of a control enclosure, respectively. In contrast, crayfish had only 26% methyl ^{203}Hg (±5%, $N = 3$) after 6 wk of exposure.

Although Se did not influence the final proportion of methyl to total ^{203}Hg in skeletal muscle of pearl dace (Fig. 4B), all fish species exhibited a concentration-dependent reduction in ^{203}Hg concentration due to Se. Concentrations of total ^{203}Hg in whole bodies of pearl dace (Fig. 5) and skeletal muscle (not shown) were reduced at 10 and 100 µg Se/L but not at 1 µg Se/L. Yellow perch had lower ^{203}Hg concentrations at 10 and 100 µg Se/L (Fig. 5), and at 1 µg Se/L, body burdens of ^{203}Hg were consistently lower (68 ± 3% (SE)) than in the controls. Similarly, young white suckers, which exhibited the highest concentrations of Se in whole bodies at 1 µg Se/L (Fig. 3), also showed a significant reduction in whole body ^{203}Hg at 1 µg Se/L (Fig. 5).

Mercury-203 concentrations in undifferentiated net plankton samples, snail whole bodies, and clam foot muscle were not affected by added Se. In contrast, ^{203}Hg concentrations in crayfish whole bodies (Fig. 5) and abdominal muscle (not shown) were reduced at 10 and 100 µg Se/L but not at 1 µg Se/L.

Discussion

GEOCHEMISTRY

The interrelationship of Hg and Se in the water column was dependent on the concentration of Se. Although the rate of movement of Hg to sediments was not altered at 1 and 10 µg Se/L (Fig. 1A; Table 1), the chemical form of ^{203}Hg was affected, as evidenced by the reduced amount associated with the activated charcoal fraction (nonpolar dissolved organics, NDO) (Table 2). At 100 µg Se/L the rate of movement of Hg to the sediments was increased (Fig. 1A and 1C; Table 1), confirming earlier observations (Rudd et al. 1980a).

At natural Se concentrations, i.e. in the control enclosures (<0.2 µg Se/L), ^{75}Se concentration in water appeared to stabilize after only 4 wk at one-half of initial concentrations

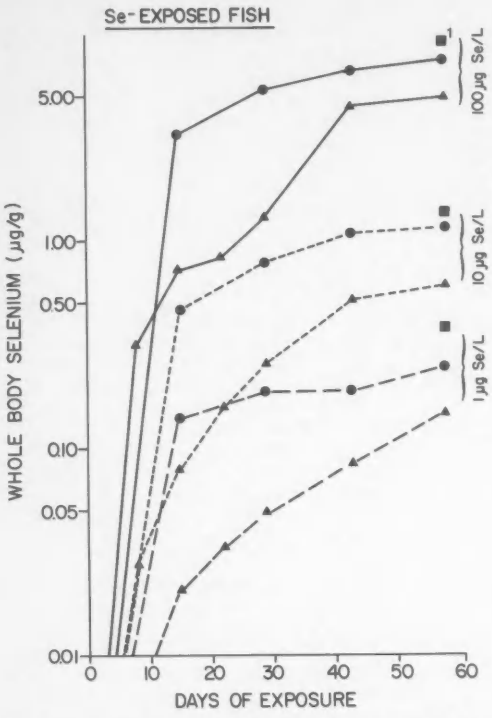


FIG. 3. Bioaccumulation of Se by juvenile white sucker (■), young-of-the-year yellow perch (●), and adult pearl dace (▲) after a single addition of 1 (– –), 10 (---), or 100 μg Se/L (—). Each point usually represents the lognormal mean of six dace, three perch, or six suckers. Only one sucker was retrieved from the 100 μg Se/L enclosure (superscript 1).

(Fig. 1B). In the controls, Se dynamics were dominated by the charcoal fraction (NDO) (Table 2). Selenium is strongly attached to this fraction because addition of Na_2SeO_3 at 1 mg Se/L failed to dislodge ^{75}Se that had been bound previously by the NDO when added at trace Se concentrations (Rudd et al. 1980b). The strength of this binding to NDO is consistent with the observation of Jackson et al. (1980) that Se is bound strongly to humic matter.

In the controls, after 2 wk, the initially large proportion of ^{75}Se on particulates (~50%) had either settled out or been redistributed among the other fractions. Subsequent stabilization of NDO-dominated Se (Table 2) between water and sediments (Fig. 1B) suggests that NDO-Se has a different removal rate than either the particulate or ionic fractions.

In the presence of elevated Se concentrations, ionic Se predominated (Table 2), probably as an anion as in the earlier work (Rudd et al. 1980a). There was a smaller percentage of NDO-Se with increasing concentration of stable Se (Table 2), declining from 27 to 19% at 1 and 100 μg Se/L, respectively. This suggests that the NDO fraction was largely saturated at 1 μg Se/L because a much larger proportion (63 ± 1%, SE) of ^{75}Se was associated with the NDO fraction in the control enclosures.

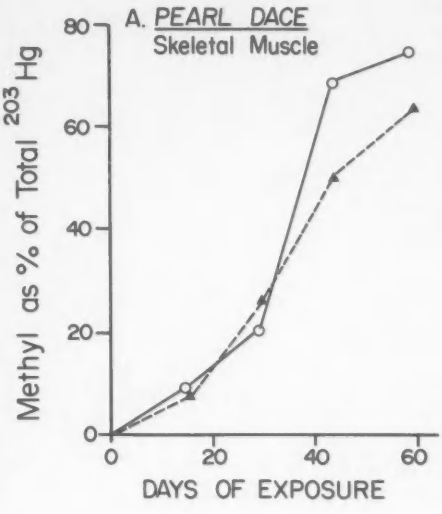
Stable partitioning of ^{75}Se between water and sediments was not apparent after 6 wk in the Se enclosures (Fig. 1B and 1D; Table 3). Although initial movement of Se to the sediments was not as rapid at elevated Se concentrations as in the controls (Fig. 1B), a greater proportion, being ionic (Table 2), would be available to move to the sediments in the long term. Observations of either longer duration or increased sediment to water ratio are required to determine the final water concentrations resulting from these levels of Se additions.

Proportionately less ^{75}Se, but not ^{203}Hg, accumulated in the haptobenthos with increasing concentration of ambient Se (Fig. 1E and 1F). The pattern of areal accumulation of radioisotopes in haptobenthos resembled that in the sediments (Fig. 1C–1F). With the progressive increase in haptobenthic biomass during the experiment, radioisotope concentrations per unit mass declined with time in all but the 1 μg Se/L enclosure (not shown). In spite of haptobenthic and sediment areas being similar (52 and 79 m^2, respectively), haptobenthos was a minor sink for added ^{203}Hg and ^{75}Se, accumulating less than 2% of either radioisotope.

The quantity of Se accumulating in the sediments was related to the level of addition. After 6 wk, ^{75}Se was confined to the upper 1-cm stratum of sediments, as compared with 3 cm in the earlier experiment (Rudd et al. 1980b). Based on an assumed eventual mixing depth of 5 cm (Schindler et al. 1977), at the rate of accumulation observed in the 1 μg Se/L enclosure (Fig. 1D), a 1-yr continued addition of Se, with a water concentration of 1 μg Se/L, would increase sediment Se by 0.08 μg/g dry wt compared with current levels of 0.4 μg/g (Rudd et al. 1980a). In contrast, after only 6 wk at 100 μg Se/L, sediment Se was increased more than 10-fold in the upper 1 cm. Additionally, a noticeable odor thought to be characteristic of Se was detected in the sediments of the 100 μg Se/L enclosure, possibly due to an organically reduced selenide (Chau et al. 1976).

BIOACCUMULATION OF SELENIUM

After addition to our experimental aquatic ecosystems, Se accumulated in all biota examined (e.g. plankton and benthos in Fig. 2, fish in Fig. 3) in proportion to ambient levels of Se, with little evidence of saturation, except perhaps for crayfish (Fig. 2B). There were, however, two distinct patterns of accumulation, probably determined by the source of Se. In net plankton, Se concentrations were greatest near the beginning of exposure, declining subsequently with time (Fig. 2) approximately following diminishing Se concentrations in water (Fig. 1B). Direct accumulation of Se from water has been demonstrated in the algae *Peridinium cinctum* (Lindstrom and Rodhe 1978) and *Scenedesmus dimorphus* (Sandholm et al. 1973). Though it is unknown to what extent zooplankton can incorporate Se by feeding on algae, the pattern of accumulation concurs with laboratory demonstrations of the ability of *Daphnia* sp. to rapidly assimilate Se compounds from water directly at concentrations from ~1 μg Se/L (Sandholm et al. 1973) to 100 μg Se/L (Schultz et al. 1980). The interval prior to attainment of maximum concen-



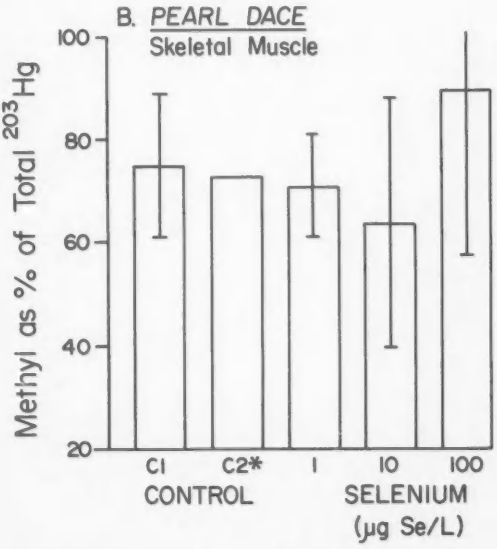


FIG. 4. Methyl ^{203}Hg as a fraction of total ^{203}Hg. (A) Temporal changes in the proportion of total ^{203}Hg present as methyl in skeletal muscle of pearl dace in a control (○) and Se (▲, 10 µg Se/L) enclosure. Each point represents the mean of three individuals. The Se and control conditions do not appear to be different. (B) Proportion of methyl ^{203}Hg in pearl dace skeletal muscle after 2 mo of exposure in the control and Se enclosures. Standard deviations about the means (N = 3) are shown. *No dace were retrieved from control 2 on day 58, so that the mean for control 2 was extrapolated from the relationship with control 1 observed after 43 d of exposure.

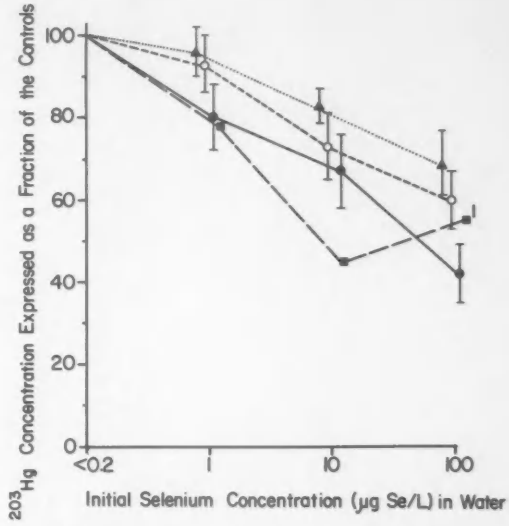


FIG. 5. Mercury-203 accumulation in whole bodies of adult crayfish (○), adult pearl dace (▲), young-of-the-year yellow perch (●), and juvenile white sucker (■) in the Se enclosures. Data are expressed as ratios in which ^{203}Hg activities in Se-exposed animals are divided by the mean activities of the combined controls. Means (±SE) of the several sampling periods (crayfish: 4, dace: 6, perch: 4, and sucker: 1) are presented for each organism. Only one sucker was retrieved from the 100 µg Se/L enclosure (superscript 1).

trations in net plankton appeared to increase from 2 d in the control and 1 µg Se/L enclosures to 8 and 14 d in the 10 and 100 µg Se/L enclosures, respectively (Fig. 2). Although this dose-dependent delay may reflect the higher concentrations being attained, *Daphnia pulex* achieved maximum concentrations within 3 d at 100 µg Se/L (Schultz et al. 1980). Possibly the biological half-life of Se in plankton is proportional to ambient Se levels.

A second general pattern was noted in the other organisms monitored, with concentrations increasing throughout the experiment or remaining stable after achieving some maximum value (e.g. crayfish in Fig. 2 and fish in Fig. 3). This pattern may be due partly to a longer biological half-life for Se in these biota. In fish at least, it could be related to homeostatic regulation of tissue Se levels (Hilton et al. 1980). This pattern is also consistent with the increased importance of food as a source of Se because of the delay inherent in incorporation of Se in food items. In marine mussels and shrimp, the distribution of ^{75}Se among organs was more similar to that of stable Se when ^{75}Se was incorporated from food rather than from water directly (Fowler and Benayoun 1976a, 1976b). Dietary sources of Se are known to be important for fish (Sandholm et al. 1973; Hilton et al. 1980; Rudd et al. 1980a), being predominant for northern pike (*Esox lucius*) at natural levels, with waterborne Se of significance only at elevated levels (Turner and Swick 1983). These observations suggest that for a pollutant monitoring program, sampling of plankton would reveal Se concentrations recently in water, while

TABLE 4. Summary of literature examining the toxicity of Se to aquatic biota.

Organism	Selenium compound tested[a]	Reference[b]	Observations
Bacteria			
Escherichia coli	SeO_3^{2-}	1	96-h LC50: 90 mg Se/L
Algae			
Chlamydomonas eugametos	?	8	100 μg Se/L caused ~70% decline in growth
Chlorella vulgaris	?	8	50 μg Se/L caused ~32% decline in growth
Haematococcus capensis	?	8	50 μg Se/L caused ~65% decline in growth
Peridinium cinctum	SeO_3^{2-}	7	In culture medium, growth at 1.6 μg Se/L was 80% of the optimum rate observed at 50 ng Se/L; but in lake water maximum growth was seen at the highest concentration tested (158 ng Se/L)
Scenedesmus	SeO_3^{2-}	1	96-h LC50: 2.5 mg Se/L
Zooplankton			
Daphnia magna	SeO_3^{2-}	5	Survival and reproduction unaffected at <0.43 mg Se/L
Daphnia pulex	SeO_3^{2-}	13	96-h LC50: fed adult, 0.44−0.57 mg Se/L (95% confidence interval); 96-h LC50: fed juvenile, 0.12−0.14 mg Se/L (95% confidence interval). Toxicity increased with duration of exposure
Daphnia	SeO_3^{2-}	1	48-h LC50: 2.5 mg Se/L
Microregma	SeO_3^{2-}	1	96-h LC50: 183 mg Se/L
Zoobenthos			
Anodonta sp.	SeO_3^{2-}	12	Increased burrowing depth at 1.0 mg Se/L inferred as avoidance behavior
Hyalella azteca	SeO_3^{2-}	5	336-h LC50: 0.07 mg Se/L. Survival and health unaffected at 0.03 mg Se/L
Lymnae stagnalis L.	SeO_3^{2-}	14	7.5-d LC50: 3 mg Se/L
	SeO_4^{2-}	14	6-d LC50: 15 mg Se/L
Xenopis laevis	SeO_3^{2-}	2	1 mg Se/L eventually lethal after 1−10 d of continuous exposure at early gastrula stage
Fish			
Brachydanio rerio	SeO_2	11b	Hatching unaffected by 0.5−10 mg Se/L. Larval mortalities <1% at 1 mg Se/L
	SeO_3^{2-}	11a	Larval 96-h LC50: 15−23 mg Se/L (95% confidence interval)
	SeO_4^{2-}	11a	Larval 96-h LC50: 81−82 mg Se/L (95% confidence interval)
Carassius auratus	SeO_2	3	Juvenile 96-h LC50: 36.6 mg Se/L
Catostomus commersoni	SeO_3^{2-}	9	96-h LC50: 29 mg Se/L
Cyprinus carpio	SeO_2	7	5 mg Se/L induced a 2−3% reduction in hatchability; 1 mg Se/L did not affect hatchability but enhanced Hg-induced ($HgCl_2$) mortality at 1 mg Hg/L
Esox lucius	SeO_3^{2-}	9	75-h LC50: 11 mg Se/L
Ictalurus punctatus	SeO_2	3	Juvenile 96-h LC50: 18.2 mg Se/L
Lepomis macrochirus	SeO_2	3	Juvenile 96-h LC50: 40.0 mg Se/L; 336-h LC1: 8.1 mg Se/L
Perca flavescens	SeO_3^{2-}	12	Over course of 1 mo continuous exposure to 1 mg Se/L, death of young-of-the-year observed
	SeO_3^{2-}	9	240-h LC50: 5 mg Se/L
Pimephales promelas	SeO_3^{2-} (?)	5	Fry 96-h LC50: 1.0 mg Se/L; 336-h LC50: 0.60 mg Se/L
	SeO_3^{2-}	3	Fry 96-h LC50: 2.9 mg Se/L. Juvenile 96-h LC50: 7.3 mg Se/L; 168-h LC1: 0.86 mg Se/L
Salmo gairdneri	SeO_3^{2-}	6	Eggs: marginal increase in mortality of eyed eggs at >28 μg Se/L. Juvenile (0.8 g) 96-h LC50: 7.2 mg Se/L; 9-d LC50: 3.7−8.0 mg Se/L (95% confidence interval). Suggested that >50 μg Se/L induced sublethal responses such as reduced blood iron and red blood cell volume
		4	96-h LC50: 2.5 mg Se/L. No mortalities observed at 0.1 mg Se/L over 28 d
Salvelinus fontinalis	SeO_2	3	96-h LC50: 14.3 mg Se/L in adults

[a] SeO_2 is a solid that when dissolved in water becomes either selenite and/or selenate (Huckabee and Griffith 1974). ?, unclear from original text which Se compound was tested.

[b] 1, Bringmann and Kuhn 1959; 2, Browne and Dumont 1979; 3, Cardwell et al. 1976; 4, Gissel Nielsen and Gissel-Nielsen 1978; 5, Halter et al. 1980; 6, Hodson et al. 1980; 7, Huckabee and Griffith 1974; 8, Hutchinson 1973; 9, Klaverkamp et al. 1983; 10, Lindstrom and Rodhe 1978; 11a, 11b, Niimi and LaHam 1975, 1976; 12, Rudd et al. 1980a; 13, Schultz et al. 1980; 14, Van Puymbroeck et al. 1982.

fish concentrations would integrate events over a longer time frame.

The rate of approach to stable concentrations in fish appeared independent of ambient Se levels (Fig. 3), as observed for *Salmo gairdneri* when Se was available at 10–400 μg Se/L (Gissel Nielsen and Gissel-Nielsen 1978). Although stable levels of Se were not achieved after 2 mo, final values varied consistently among the experimental fish species with adult pearl dace < young-of-the-year yellow perch < young white sucker (Fig. 3). Because Se concentrations in skeletal muscle varied little among six fish species in Clay Lake (0.14 ± 0.01 (SE) μg Se/g, unpublished data), it is possible that the differences in levels observed among the experimental fish species largely reflect more rapid accumulation in younger fish, as has been noted for Hg (reviewed by Armstrong 1979; Huckabee et al. 1979).

Aquatic Toxicity of Selenium

Algae and zooplankton are the organisms most likely to be sensitive to increased levels of Se (Table 4). Selenium was not observed to be toxic to zooplankton in our experiment (A. Salki, Freshwater Institute, Winnipeg, Man., personal communication). Although the responses of the phytoplankton and zooplankton communities in these enclosures are considered in detail elsewhere (A. Salki, unpublished data), we can make several observations about other aspects of Se toxicology. Despite a change in sediment characteristics after 2 mo of exposure to 50–100 μg Se/L (see earlier discussion), no mortalities or obvious changes in behavior attributable to Se were noted in fish (young perch and suckers and adult dace) or in adult crayfish. This is consistent with laboratory assays of Se toxicity to fish (Table 4), where the 96-h LC50 of the most sensitive stage and species tested (fry of *Pimephales promelas*) was observed to be 1 mg Se/L, with the critical concentration declining somewhat with increased length of exposure. However, the upper limit for Se in aquatic ecosystems may more likely be determined by concern for lower forms of aquatic biota or by concern for consumers of Se-elevated biota rather than by consideration of levels demonstrated to be toxic to fish. For example, final whole body concentrations in experimental fish in the 10 μg Se/L enclosure (Fig. 3), a concentration that poses no apparent threat to fish (our observations; Table 4), were nearly threefold higher than the regional mean concentration of 0.5 μg Se/g in whole bodies of fish in the Great Lakes (Beal 1974).

Influence of Selenium on Mercury Bioaccumulation

The ability of Se to reduce the bioaccumulation of Hg in biota is dose dependent. The observation that 100 μg Se/L reduced accumulation of Hg (Rudd et al. 1980a) was confirmed in pearl dace and crayfish and extended to yellow perch and white sucker in this study (Fig. 5). A smaller reduction in ^{203}Hg accumulation by these species was observed at 10 μg Se/L (Fig. 5). At 1 μg Se/L only white suckers and yellow perch, which had accumulated Se to the greatest extent (Fig. 3), showed a significant reduction in ^{203}Hg concentration (Fig. 5). At all levels of added Se, whole body concentrations of Se increased consistently from dace to perch to sucker (Fig. 3). A similar relationship between Se accumulation and Hg levels was observed by Speyer (1980) in pike of two similar lakes in Quebec affected by mining concentrations with elevated levels of Hg in the surficial sediments. Pike in Lake Duparquet, with Se concentrations ranging from <0.2 to 0.6 μg Se/g wet wt, had Hg levels from 0.2 to 1.4 μg Hg/g. In contrast, in Lake Dufault, pike with elevated levels of Se (1.1–3.0 μg Se/g) exhibited reduced levels of Hg (0.01–0.03 μg Hg/g). Thus, the extent to which bioaccumulation of ^{203}Hg decreases in response to Se treatment appears to be proportional to the amount of Se bioaccumulated by that fish.

The position of the organism in the food web appears to be important in determining whether added Se will reduce bioaccumulation of Hg. In addition to lowered accumulation of ^{203}Hg in fish (Fig. 5), bioaccumulation of ^{203}Hg was reduced in predaceous megaloptera and chaoborids in related observations (B. E. Townsend, Freshwater Institute, Winnipeg, Man., personal communication). Reductions were not seen in detrivorous insects (B. Townsend, personal communication), clams, snails, and mixed plankton populations (this study, not shown). At the lowest levels in the food web, there is little or no Se effect, but at higher ones monitored in these enclosure ecosystems, a reduction in accumulation of Hg was apparent. One would predict a greater reduction in Hg accumulation to occur in predaceous fish at low Se concentrations than in the detrivorous and omnivorous fish used in these experiments, and this is found to be so (Turner and Swick 1983).

Selenium as an Aquatic Mercury Ameliorating Agent

Selenium can alleviate Hg poisoning in fish consumers (e.g. Chang et al. 1977) and can reduce accumulation of Hg in aquatic food webs (Rudd et al. 1980a, Fig. 5; Turner and Swick 1983). Because of these attributes, it has potential utility as a Hg ameliorating agent in both point-source and atmospheric transport problems. Some of the beneficial effects (e.g. Fig. 5) that we have observed, and some potential toxic effects, may be occurring now in aquatic ecosystems as a result of the atmospheric transport of Se. The effects of such deposition on the aquatic environment could vary according to the relative quantities of Hg and Se discharged to the atmosphere as well as to the relative distance of atmospheric transport. These effects could range from toxicity problems near large atmospheric sources to ameliorative effects at distances where Hg and Se deposition rates are similar.

In terms of a point-source problem, it is useful to consider the specific example of the Hg-polluted Wabigoon River system (Armstrong and Hamilton 1973; Parks et al. 1980). Selenium concentrations in aquatic biota of this system are low (see earlier discussion). Consequently, fish Hg:Se ratios appear to be the highest for which we have knowledge. This unfortunate situation might be partially remedied by the addition of low levels of Se. Although the single experimental addition of 1 μg Se/L did not always reduce the rate of ^{203}Hg bioaccumulation in nonpredatory fish (Fig. 5), with the food web enhancement observed in Turner and Swick (1983), Hg concentrations in predatory fish (fish species most often consumed by humans) may be reduced at 1 μg Se/L. At least there should be substantial improvement in the Hg:Se ratio, reducing the toxicity of Hg in fish at its present concentration.

At best, there would also be a reduction in Hg concentration in all fish.

If it were decided to add Se to the Wabigoon River system, it should be possible to increase concentrations of Se threefold in skeletal muscle of fish of this system to around 0.5 μg/g. This value is nearer to the more typical concentrations found presently in fish muscle at the nearby Experimental Lakes Area (0.48 ± 0.13 (SE) μg Se/g, three species, unpublished data) and in whole bodies of fish in the Great Lakes (Adams and Johnson 1977; Armstrong and Lutz 1977a, 1977b; Beal 1974). In our experiments this was almost achieved in young-of-the-year yellow perch and juvenile white suckers in the 1 μg Se/L enclosure (0.24 and 0.38 μg/g, respectively, Fig. 3) after only 2 mo of exposure. Thus, over the lifetime of a fish, it is possible that a concentration in water of less than 1 μg Se/L would be required. Because Se is accumulated efficiently through the food web (Rudd et al. 1980a; Turner and Swick 1983), it should not be necessary to maintain Se at an elevated concentration in water for extended periods. Additions of Se could be made as required as determined by close chemical monitoring of representative species and environmental compartments.

The use of Se as a Hg ameliorating agent should be approached cautiously because of its possible toxicity to aquatic biota (reviewed in Table 4). However, with a treatment concentration of less than 1 μg Se/L, a substantial safety margin for most aquatic biota is likely. Toxicity information currently available (Table 4) indicates that 1 μg Se/L is substantially lower than known chronic sublethal concentrations for the most sensitive aquatic biota tested (*Daphnia pulex*). A level of 1 μg Se/L is significantly below the lower lethal limit of the most sensitive aquatic vertebrate tested (*Pimephales promelas*). Moreover, fish at least appear able to regulate Se bioaccumulation (Hilton et al. 1980; Hodson et al. 1980; Turner and Swick 1983). A further safety feature is that downstream of Clay Lake, elevated Se concentrations would be substantially diluted at Ball Lake, the confluence of the Wabigoon and English River systems, where concentrations of Hg in biota at Ball Lake are less than those characteristic of Clay Lake biota (Armstrong and Hamilton 1973; Parks et al. 1980). Consequently, both availability and risk of the added Se would be proportional to need.

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The English–Wabigoon River System: IV. Interaction between Mercury and Selenium Accumulated from Waterborne and Dietary Sources by Northern Pike (*Esox lucius*)

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Although selenium is a pollutant released by several industries, it is also an essential nutrient that protects mammals against mercury intoxication. When added to aquatic ecosystems, selenium is bioaccumulated readily and can reduce mercury accumulation in some biota. Using a predator–prey experimental model, we investigated both the route of selenium uptake and the mechanism of reduction in mercury accumulation. Young northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) were caged in situ in mercury-contaminated Clay Lake, northwestern Ontario. Pike were held in water containing trace (<0.2 μg Se/L) or elevated (4.5–6.4 μg Se/L) concentrations of selenium and were able to accumulate ^{203}Hg and ^{75}Se from food (yellow perch) only, water only, or from food and water. Control pike accumulated as much as 20 times more ^{75}Se from food than from water, assimilating ~30% of selenium in food. With increased levels of selenium in water (around 5 μg Se/L), food and water were of similar importance as sources. Waterborne selenium did not alter either the amount of ^{203}Hg accumulated from water or its subsequent partitioning among the pike tissues sampled. When elevated in food, selenium decreased both the body burden of ^{203}Hg in pike and the proportion in muscle. It is inferred that selenium added to aquatic ecosystems, and incorporated subsequently in the food web, would interfere with biomagnification of mercury. Furthermore, future studies of selenium toxicity in fish should emphasize its accumulation from food.

TURNER, M. A., AND A. L. SWICK. 1983. The English–Wabigoon River system: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (*Esox lucius*). Can. J. Fish. Aquat. Sci. 40: 2241–2250.

Bien que le sélénium soit un contaminant libéré par plusieurs industries, il est en même temps un nutriment essentiel protégeant les mammifères contre l'intoxication par le mercure. Ajouté au système aquatique, le sélénium est facilement bioaccumulé et peut diminuer, dans certaines biocénoses, l'accumulation du mercure. À l'aide d'un modèle expérimental prédateur–proie, nous avons étudié à la fois la voie d'assimilation du sélénium et le mécanisme de réduction de l'accumulation de mercure. De jeunes brochets (*Esox lucius*) et perchaudes (*Perca flavescens*) ont été placés dans des cages in situ dans le lac Clay, dans le nord-ouest de l'Ontario, contaminé par le mercure. Les brochets ont été maintenus dans de l'eau contenant du sélénium à l'état de traces (<0,2 μg Se/L) ou de fortes concentrations (4,5–6,4 μg Se/L), et ils ont pu accumuler ^{203}Hg et ^{75}Se de la nourriture seulement (perchaudes), de l'eau seulement, ou de la nourriture et de l'eau. Les brochets témoins accumulent jusqu'à 20 fois plus de ^{75}Se de la nourriture que de l'eau, assimilant ~30 % de sélénium de la nourriture. Avec des augmentations de sélénium dans l'eau (environ 5 μg Se/L), la nourriture et l'eau sont d'importance égale comme sources. Le sélénium contenu dans l'eau ne modifie ni la quantité de ^{203}Hg accumulé à partir de l'eau, ni sa répartition subséquente parmi les tissus des brochets échantillonnés. Lorsque présent en plus fortes concentrations dans la nourriture, le sélénium diminue à la fois la charge corporelle de ^{203}Hg chez les brochets et la proportion dans le muscle. On en déduit que le sélénium ajouté aux écosystèmes aquatiques et incorporé subséquemment dans le réseau alimentaire interférerait avec la bioamplification du mercure. En outre, on devrait, lors d'études futures sur la toxicité du sélénium chez les poissons, souligner son accumulation à partir de la nourriture.

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SELENIUM is both an essential nutrient and an industrial pollutant (reviewed by DeMayo et al. 1979 and Vokal-Borek 1979). When added to aquatic ecosystems at either trace (<0.2 μg Se/L) or higher (1−100 μg Se/L) concentrations, Se can move rapidly from water to sediments (Hesslein et al. 1980; Jackson et al. 1980; Rudd et al. 1980; Turner and Rudd 1983). Aquatic biota accumulate Se efficiently at concentrations in water ranging from <0.2 to 100 μg Se/L (Rudd et al. 1980; Turner and Rudd 1983). Although aquatic biota can accumulate both inorganic and organic Se compounds directly from water (Sandholm et al. 1973; Hodson et al. 1980), laboratory aquarium experiments indicate that food is also important (Sandholm et al. 1973). Experiments in large enclosures also suggest that food may be the dominant source of Se for aquatic biota (Rudd et al. 1980). However, experimental demonstration of the relative importance of food and water for Se uptake in natural ecosystems is lacking.

Careful addition of Se to watercourses, at low concentrations, has been suggested as an ameliorative procedure for systems contaminated by point-source discharges of Hg (Rudd et al. 1980; Turner and Rudd 1983). When present in food contaminated by Hg, Se may decrease or prevent Hg poisoning in mammals, in spite of frequently increasing the retention of Hg (Ganther et al. 1972; Chang et al. 1977; Friedman et al. 1978). In short-term aquarium experiments, Kim et al. (1977) pretreated northern creek chubs (*Semotilus atromaculatus*) with SeO_2 added to water (3 mg Se/L). Thereafter, chubs were protected from otherwise toxic concentrations of inorganic Hg (>70 μg Hg/L), but the relationship between Hg concentrations in water and subsequent accumulation of Hg was erratic. Se concentrations of 10 and 100 μg Se/L in water, added initially as Na_2SeO_3, reduced Hg accumulation in pearl dace (*Semotilus margarita*) and crayfish (*Orconectes virilis*) in aquatic ecosystem experiments (Rudd et al. 1980; Turner and Rudd 1983). Selenium also reduced accumulation of ^{203}Hg in white suckers (*Catostomus commersoni*) and possibly did so at 1 μg Se/L in yellow perch (*Perca flavescens*) (Turner and Rudd 1983). Although 100 μg Se/L increased the rate of movement of ^{203}Hg from water to sediments, neither sedimentation nor chemical speciation of ^{203}Hg was much changed at 1 or 10 μg Se/L (Turner and Rudd 1983). The mechanisms underlying the influence of Se on Hg bioaccumulation remain unclear.

Our objective was to assess the relative importance of water and food in the bioaccumulation of Hg and Se by predaceous fish at both trace and elevated concentrations of Se in water and to determine whether added Se would decrease the transfer of Hg between trophic levels. This project was conducted as part of a research program on the Hg-contaminated Wabigoon River system (Rudd et al. 1980, 1983).

Methods

To assess the influence of elevated Se on accumulation of Hg and Se from both food (yellow perch) and water by northern pike (*Esox lucius*), fish were held in cages in a sheltered bay of Clay Lake, northwestern Ontario (50°03′N, 93°30′W; Armstrong and Hamilton 1973). These experiments began Aug. 15, 1979, approximately 1 mo after the beginning of the Hg amelioration by Se experiment (Turner and Rudd 1983). In one experiment, cages were positioned within water-tight polyethylene-walled cylinders (1.7 m deep, 10 m in diameter, and ~130 m^3 volume) which enclosed lake water and were sealed into the sediments. The enclosures are described further in Turner and Rudd (1983). Five milliCuries of $^{203}Hg(NO_3)_2$ and 3 mCi of $H_2{}^{75}SeO_3$ (1 Ci = 37 GBq; New England Nuclear) were added initially to a control enclosure. Sufficient carrier was added with the ^{203}Hg to yield a concentration of about 15 ng Hg/L after dilution in the enclosures. The final concentration of Se after dilution of the added ^{75}Se and carrier Se was below the analytical detection limit of 0.2 μg Se/L. A second enclosure also received $^{203}Hg(NO_3)_2$, but the $H_2{}^{75}SeO_3$ was mixed previously with sufficient Na_2SeO_3 to yield an initial concentration of 10 μg Se/L. Methods for collection of water samples, their fractionation, and radioassay are presented in Rudd and Turner (1983).

To quantify accumulation of Hg and Se from food and water, predators (young northern pike) were exposed to ^{203}Hg and ^{75}Se in food (young-of-the-year yellow perch) and water or in water alone (Fig. 1A). In a corroborative experiment, pike were exposed to radioisotopes through consumption of radiolabeled perch in the absence of radioisotopes in the surrounding water (Fig. 1B). Each experiment had two groups: a control, with a trace amount of Se (<0.2 μg Se/L), and another group to which Se was added.

Fish for these experiments were obtained by seining along a beach in Clay Lake. Northern pike of ~35 g and yellow perch of ~2 g were selected and assigned randomly to experimental treatments.

In the main experiment, three fish cages were placed in each 10-m-diameter enclosure (Fig. 1A). Cages (1.2 × 1.2 × 1.2 m) were constructed with wooden frames and untreated Ace-style nylon netting with 0.64-cm mesh (Nichols Net and Twine Co.). In each of the control and added Se conditions, there were two groups of pike. All pike in this experiment were exposed to waterborne ^{75}Se and ^{203}Hg, but one group of pike could also consume radiolabeled perch enclosed in the same cage. After 1 and 2 wk, three pike were taken from each treatment enclosure and radioassayed for whole body and tissue radioisotope concentrations. In addition, radioisotope concentrations in whole bodies of three perch were determined for each combination of Se (control or added Se) and duration (1-, 2-, or 3-wk exposure). Perch were placed in cages 1 wk prior to addition of the pike and could accumulate radioisotopes from both water and food (e.g. zooplankton). The nominal food ration for each pike was four perch per week. The number of perch remaining in each cage at the end of a sampling period was determined so that the ration could be adjusted accordingly.

In the corroborative experiment, two additional cages were placed outside the 10-m-diameter enclosures, each within a larger cage of wire mesh (Fig. 1B). In absence of exposure to radioisotopes in the surrounding water during this experiment, pike were fed perch that had been labeled previously with ^{203}Hg and ^{75}Se, in cages within the control and elevated Se enclosures. The nominal ration was four perch per pike. Selenium levels in perch in the Se-treated group were elevated compared with controls by approximately 0.8 μg Se/g (wet wt). Pike were collected 6 d after addition of the perch and sampled as in the earlier experiment.

Fish were frozen using dry ice immediately after sampling. Samples were kept frozen until dissection into several tissues: dorsal skeletal muscle, liver, gill (including arch), skin, gastrointestinal musculature, and carcass (whole body excluding other tissues). Gastrointestinal contents were extruded from the gut and collected separately but were not included in estimates of either whole body concentrations or radioisotope consumed. The gut was rinsed with tap water and prepared separately for radioassay. Samples were then refrozen until analysis.

Radioisotope concentrations in whole bodies of pike, excluding gastrointestinal contents, were determined from concentrations in observed tissues and carcass samples. Radioisotope concentrations in whole bodies of pike and perch, and proportions of radioisotopes in tissue of pike, were examined using factorial analysis of variance (Snedecor and Cochran 1967). Otherwise, lognormal means and variances are presented for each group of pike or perch.

"Assimilation efficiency" (Aoyama and Inoue 1973; Phillips and Gregory 1979) was calculated to determine the efficiency of net accumulation of each radioisotope from food. This accounts for different amounts of radioisotope in perch of the Se-treated and control groups:

$$\text{Assimilation efficiency} = \frac{\text{(total radioisotope consumed)} - \text{(egestion)}}{\text{(total radioisotope consumed)}}$$

$$= \frac{\text{(residual radioisotope in body of pike)}}{\text{(total radioisotope consumed)}}$$

$$= \frac{\text{(mean weight of all pike)} \times \text{(mean concentration of radioisotope in whole body of pike obtained from food}^{1}\text{)}}{\text{(mean No. of perch consumed/pike}^{2}\text{)} \times \text{(mean radioisotope burden in perch whole bodies}^{3}\text{)}}.$$

Calculations were made for each group of three pike and not for individuals, because the amount of radioisotope ingested by each group was known better than that of an individual. The assimilation efficiency reported for week 2 represents a mean for the 2-wk period.

Results

SELENIUM

In the control enclosure, concentration of ^{75}Se in the water column was relatively constant at approximately 1125 cpm/L during the 3 wk of the experiment (Fig. 2A). In the Se enclosure, ^{75}Se moved to the sediments during the experiment (Turner and Rudd 1983) declining from 1275 to 900 cpm/L in water, with corresponding total Se concentrations of ~6.4 and 4.5 μg Se/L.

The chemical partitioning of Se in water was also different between these two enclosures. In the control enclosure, ~60% of ^{75}Se was associated with nonpolar dissolved organic substances and about 25 and 15% in ionic or particulate forms. In the Se enclosure, ionic species predominated (~70%), with the remainder associated largely with nonpolar dissolved organics.

Selenium-75 in perch increased rapidly throughout the 3-wk period in both control and treatment conditions, although there was a suggestion of a lag in uptake during the 1st wk of exposure, especially noticeable in the Se-treated enclosure (Fig. 2B). Differences in specific activity (^{75}Se:Se) between the control and Se enclosures prevent direct comparison of perch ^{75}Se concentrations.

In the control condition, ^{75}Se concentrations in pike allowed to feed on ^{75}Se-labeled food were 7 (week 1) and 20 times (week 2) greater ($P < 0.001$) than when ^{75}Se was supplied only in water (Fig. 3A, control). With increased concentrations of Se in water, the relative importance of water as a source of ^{75}Se increased (Fig. 3A, Se). Although the concentrations of ^{75}Se in the perch were reduced (Fig. 2B), assimilation of Se from foodstuffs was reduced in the presence of increased concentrations in water (Fig. 3B, Se: food + water vs food). In the control, assimilation of Se from food (perch) was approximately 30%, whereas assimilation ranged from 8 to 19% with the addition of Se (Fig. 3B, food + water: control vs Se). When Se was increased in food but not supplied in water, as in the corroborative experiment (Fig. 1B), assimilation was not very different from that in the control (Fig. 3B, food only).

Partitioning of Se among the tissues sampled, as indicated by tissue to whole body concentrations (Table 1), was not altered greatly by either the amount of Se present (control vs Se) or the route of Se uptake (water vs food and water). In all treatments, the proportion of ^{75}Se was highest in the liver (5.8–15.0) and lowest in the skeletal musculature (0.21–0.41). The proportion of ^{75}Se in the gut musculature was somewhat higher in pike fed ^{75}Se-labeled food than in pike with access to ^{75}Se in water only ($P < 0.01$). This increase was reduced in the presence of elevated Se concentrations in water (Table 1), which is consistent with the observation of reduced assimilation (Fig. 3B, food + water: control vs Se).

[1]Accumulations of radioisotopes from food and water were assumed to be independent processes as observed for Hg in rainbow trout (*Salmo gairdneri*) (Phillips and Buhler 1978). Mercury-203 and ^{75}Se observed to accumulate in the "water-only" group were subtracted from the "food plus water" group to yield an estimate of the contribution due to food alone.

[2]When corrected for perch remaining in the cage and material remaining in the gut, calculated rations for individual control pike were 3.3 and 3.9 perch/wk in weeks 1 and 2, respectively. Corresponding rations for Se-exposed pike were 2.2 and 3.3 perch/wk.

[3]Weekly radioisotope concentrations in perch were calculated as the arithmetic mean of the concentrations observed at the beginning and end of that week. No correction was made for radioisotopes remaining in the gastrointestinal system of the perch.

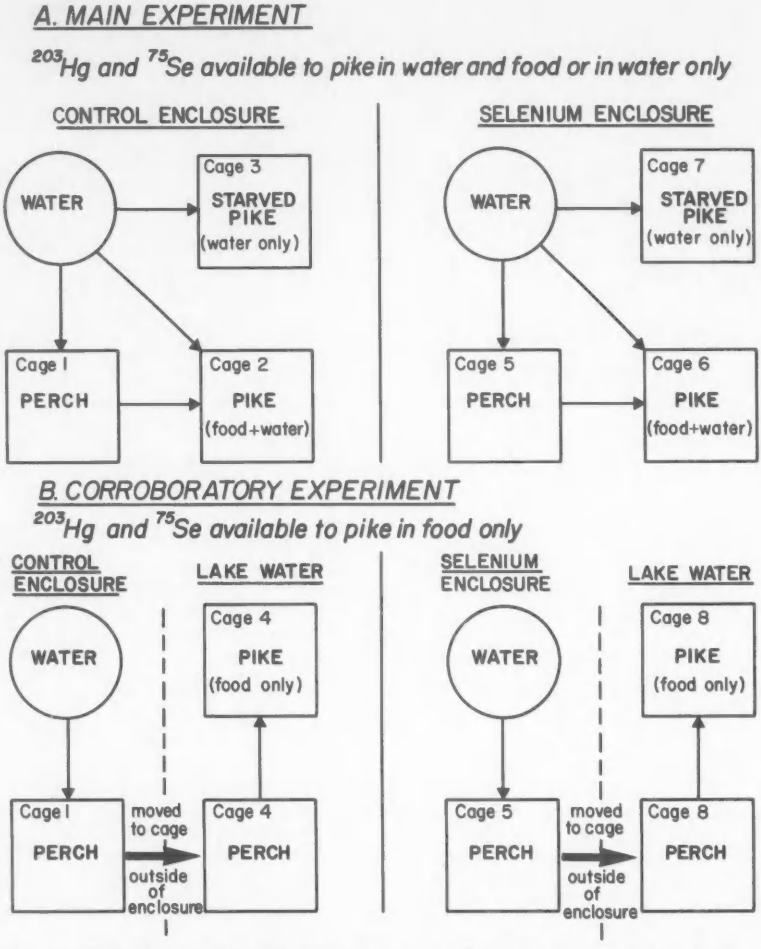


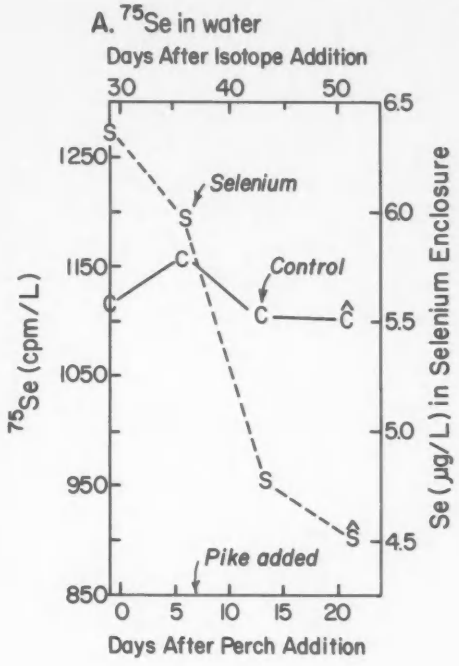
FIG. 1. Schematics of experimental designs. In the main experiment (A), pike can accumulate ^{203}Hg and ^{75}Se from food and water, or from water only, in the presence or absence of added Se. In the corroborative experiment (B), radioisotopes are accumulated only by consumption of prey (perch). Arrows represent main routes of radioisotope movement. It is probable that perch accumulated radioisotopes from unknown food sources as well as from water directly.

MERCURY-203

Concentrations of ^{203}Hg in water were similar in both control and Se enclosures, decreasing to one-half during the experiment (Fig. 4A). There were no substantial differences in partitioning of ^{203}Hg among the different chemical fractions: particulate (>0.45 μm), nonpolar dissolved organics, ionic, and undefined.

Throughout the main experiment (Fig. 1A), ^{203}Hg in whole bodies of perch continued to increase in both the control and added Se enclosures (Fig. 3B). Mercury-203 concentrations in perch exposed to Se were consistently lower than in control perch ($P \sim 0.02$).

Where both Hg and Se radioisotopes could be taken up only from water, ^{203}Hg concentrations in whole bodies and skeletal muscle of pike were not different between the control and Se conditions (Fig. 5A, water only). Where ^{203}Hg could also be accumulated by consumption of perch, concentrations in whole bodies ($P < 0.03$) and skeletal muscle ($P \sim 0.001$) of pike were always lower in the presence of added Se (Fig. 5A, food + water). Although this is partly due to the lower amounts of ^{203}Hg ingested by Se-exposed pike relative to control pike, increased Se reduced the assimilation of ^{203}Hg from food from approximately 15% in the control to 5–11%



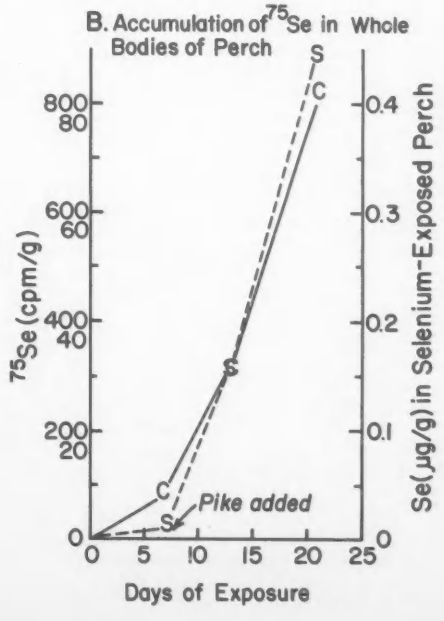


FIG. 2. (A) Selenium-75 concentration in the water columns of the control and Se enclosures during the periods when fish were exposed to ^{75}Se. Concentrations shown for the last point are predictions from models fit to earlier data (explained in Turner and Rudd 1983). (B) Selenium-75 concentrations in whole bodies of perch held in the control (C) and Se (S) enclosures. Each point represents the antilog of the lognormal mean of three individuals. Along the radioisotope axis (left-hand axis), upper and lower values refer to perch in the control and Se conditions, respectively. Given the known specific activity of ^{75}Se:Se in the Se enclosure, computed amounts of Se accumulated are also shown (right-hand axis).

(Fig. 5B, food + water). In the food-only experiment (Fig. 1B, radioisotopes and added Se present only in food), a reduction in assimilation efficiency of ^{203}Hg of similar magnitude was also observed (8 vs 14%, Fig. 5B, food only), suggesting that Se in food rather than Se in water was responsible.

Where ^{203}Hg could be taken up only from the water column, waterborne Se did not influence partitioning of ^{203}Hg among pike tissues (Table 2), not even in gill tissue. In contrast, where ^{203}Hg could also be accumulated from food, the proportion of ^{203}Hg in both the gut ($P < 0.001$) and skeletal musculature ($P \sim 0.001$) was higher than where ^{203}Hg could be taken up only from water. These increases of ^{203}Hg in gut and skeletal musculatures observed in the control condition were reduced with exposure to added Se ($P < 0.01$ and $P < 0.05$, respectively) in food and water.

Discussion

For several reasons we used radioisotopes and did not measure changes in concentration of the stable elements. The initial variability of stable Se and Hg concentrations in the perch and pike would have hindered detection of treatment effects. With addition of the radioisotopes ^{203}Hg and ^{75}Se, a definite time zero existed beyond which new accumulations of radioisotopes could be compared between test and control conditions. Using the analytical sensitivity of radioisotope techniques, we were also able to avoid the confusing effects of a large chemical addition of Hg. Given the relatively long biological half-life of Hg in fish (reviewed by Huckabee et al. 1979) it is unlikely, however, that added ^{203}Hg would have equilibrated during the experiment with Hg previously in the fish. The biological half-life of Se is less well known, although it has been determined to be approximately 1 mo in *Salmo gairdneri* (Gissel Nielsen and Gissel-Nielsen 1978). Therefore, it is also unlikely that added ^{75}Se would have equilibrated with Se previously in the pike and perch. Only in the elevated Se condition was ^{75}Se used as a radiotracer. Otherwise, we used a comparative approach and not a radiotracer strategy. By ensuring that both the radioisotope additions and the initial amounts of stable elements in the control and experimental compartments were identical, we could compare accumulations of each radioisotope between control and test conditions.

Selenium Bioaccumulation

Selenium is accumulated from both food and water (Fig. 3A and 3B). At low concentrations of Se in water (<0.2 µg

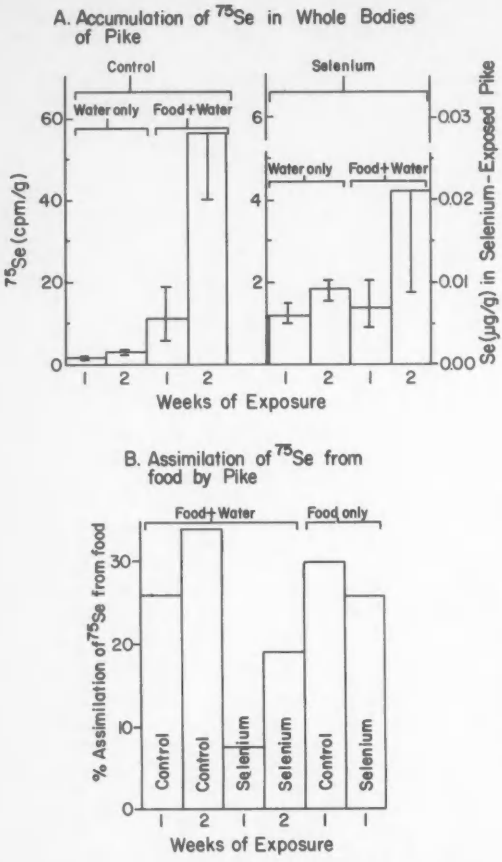


FIG. 3. (A) Whole body ^{75}Se in northern pike held for 1 or 2 wk in the control or Se enclosures. Pike were able to accumulate ^{75}Se from food (perch) and water, or from water only. Two standard error bars are shown about each mean of three individuals. The right-hand axis is as described in Fig. 2B. (B) Efficiency of assimilation of ^{75}Se from food (perch) by pike after 1 and 2 wk. To obtain the estimate of the body burden of ^{75}Se due to food alone, the uptake of ^{75}Se from water, as measured in the water-only condition (Fig. 3A), was subtracted from the body burdens observed in the food and water condition. In the corroborative, food-only experiment, the contribution of radioisotopes in food to the body burden was observed directly.

Se/L), food web transfers predominate for fish (Fig. 3A; Rudd et al. 1980; Sandholm et al. 1973), although concentrations in other organisms, such as in zooplankton, depend on water concentrations (Turner and Rudd 1983). At increased Se concentrations in water (Fig. 2A, 4.5–6.4 µg Se/L), pike accumulated only somewhat less ^{75}Se than in the control condition (Fig. 3A, water only: control vs Se), despite the fact that the ambient concentration of radioisotope was lower (Fig. 2A). The greater than 25-fold increase in Se concentration in water (<0.2 vs ~5 µg Se/L) indicates that substantially more Se was accumulated from water by Se-exposed pike.

Fish seem able to regulate the amount of Se bioaccumulated to some extent (Hilton et al. 1980; Hodson et al. 1980). Although assimilation of Se appears to be independent of food Se concentration (Fig. 3B, food only), increased concentrations of Se in water can reduce assimilation of Se (Fig. 3B, food + water: control vs Se).

Selenium concentrations vary among tissues (Table 1; Hodson et al. 1980; Turner and Rudd 1983) and partitioning can vary with time as shown in Table 1. Highest concentrations are observed in the liver (Table 1) and kidney (Hilton et al. 1980; Hodson et al. 1980), both of which have been postulated to be involved with Se regulation and excretion (Hilton et al. 1980).

Interaction between Selenium and Mercury

Caged yellow perch, which accumulated radioisotopes from both water and food (e.g. zooplankton), consistently accumulated less ^{203}Hg when exposed to added Se ($P \sim 0.02$, Fig. 4B). This was also seen in pearl dace, white suckers, and uncaged perch living in the 10-m-diameter enclosure with an initial water concentration of 10 µg Se/L (Turner and Rudd 1983). When Se was increased only in the water column (4.5–6.4 µg Se/L, Fig. 2A), there was no change in either the amount of ^{203}Hg that pike accumulated from water (Fig. 5A, water only) or the distribution of ^{203}Hg among tissues examined (Table 2). Because the proportion of methyl to total Hg in water was probably small, ~5% (Parks et al. 1980), this effect may also be seen as a lack of action of Se on uptake of inorganic Hg from water.

Control pike, able to accumulate ^{203}Hg from both water and food, obtained from 33% (week 1) to 43% (week 2) of their ^{203}Hg body burden from food (Fig. 5A, control: water only vs food + water). In both weeks, an assimilation efficiency of ~15% was observed in the transfer of ^{203}Hg from perch (food) to pike (Fig. 5B, control). This is similar to the observation by Phillips and Gregory (1979) in which pike assimilated 19% of naturally occurring methyl mercury in young-of-the-year carp (*Cyprinus carpio*). In contrast, when Se concentrations were increased in perch by 0.05–0.4 µg Se/g (Fig. 2B), assimilation of ^{203}Hg from food declined to between 5 and 11% (Fig. 5B, food and water: Se). This was corroborated by the food-only experiment (Fig. 5B, food only: Se) in which Se in perch was elevated by about 0.8 µg Se/g (wet wt) and assimilation efficiency was 8 vs 14% in the controls. These short-term observations imply that the reduction in bioaccumulation of ^{203}Hg by fish, observed in in situ ecosystem experiments (Rudd et al. 1980; Turner and Rudd 1983), is due to interference of Se with Hg mobilized through food webs, rather than on Hg accumulated directly from water. Observations of longer duration are required to determine whether the increase from week 1 to week 2 in percentage assimilation of ^{203}Hg from food in Se-exposed pike (Fig. 5B, food and water: Se) represents a trend. Because a Se-induced concentration-dependent reduction in Hg accumulation in several fish species was observed after 2 mo (Turner and Rudd 1983), we think this is unlikely.

Control pike had proportionately more ^{203}Hg in the gut and

TABLE 1. Ratios of tissue to whole body concentrations of ^{75}Se (mean ± SD, $N = 3$).

Tissue	Experimental condition	Week 1		Week 2	
		Water	Food + water	Water	Food + water
Gill	Control	5.98 (±1.56)	4.37 (±1.04)	4.31 (±0.74)	3.25 (±0.19)
	Se	5.66 (±2.49)	6.44 (±1.17)	4.86 (±2.12)	4.81 (±2.45)
Skin	Control	1.85 (±0.60)	2.16 (±0.13)	1.67 (±0.52)	1.57 (±0.17)
	Se	Not measured	0.97 (±1.37)[a]	0.80 (±0.53)	1.15 (±1.01)
Gut	Control	1.77 (±0.92)	4.05 (±0.87)	1.98 (±0.65)	3.10 (±0.65)
	Se	2.29 (±1.03)	2.82 (±0.15)	2.02 (±0.76)	1.80 (±0.35)
Liver	Control	7.80 (±3.24)	7.37 (±1.22)	9.27 (±2.76)	8.51 (±0.89)
	Se	10.84 (±3.24)	5.76 (±5.29)	14.96 (±7.51)	8.75 (±0.89)
Muscle	Control	0.21 (±0.15)	0.23 (±0.04)	0.34 (±0.07)	0.38 (±0.13)
	Se	0.41 (±0.35)	0.27 (±0.24)	0.21 (±0.19)	0.40 (±0.04)

[a] $N = 2$.

skeletal musculatures when accumulating ^{203}Hg from food and water together than from water only (Table 2). This is consistent with the observation that Hg burdens obtained via food and water are additive (Phillips and Buhler 1978). Partitioning the ^{203}Hg in muscle according to source indicates that 75–81% of the muscle ^{203}Hg burden in food- and water-exposed pike was obtained from food in weeks 1 and 2. With increased concentrations of Se in both food and water, there was a larger proportion of ^{203}Hg found in the liver in week 1 ($P \sim 0.01$), with comparatively less in the skeletal ($P < 0.05$) and gut musculatures ($P < 0.01$; Table 2, food + water: control vs Se, week 2). In contrast with control pike, in weeks 1 and 2, only 44 and 52% of ^{203}Hg in muscle of Se-exposed pike was acquired from food. Furthermore, liver ^{203}Hg probably was inorganic (Burrows and Krenkel 1973), so that the comparison between ^{203}Hg burdens of control and Se-exposed pike (Fig. 5A) could underestimate the influence of Se on body burdens of methyl mercury. The liver was also a site for high Se concentrations (Table 1). This concurs with the suggestion that the liver is a storage organ for Se in *Salmo gairdneri* (Hilton et al. 1980).

Losses of ^{203}Hg from pike were not measured so that it is unknown whether the Se-induced reduction in assimilation of ^{203}Hg (Fig. 5B, control vs Se) is due to lowered absorption of ^{203}Hg from the gut or to its enhanced depuration. However, the proportion of ^{203}Hg in gut musculature of Se-fed pike was reduced relative to controls ($P < 0.01$, Table 2, food and water). Because the source of exposure, food or water, appears unimportant to the tissue distribution of methyl mercury, except perhaps for the tissue involved in its uptake (McKim et al. 1976), it is unlikely that reduced absorption from gut can be the only mechanism involved.

IMPLICATIONS FOR MERCURY AMELIORATION

Selenium supplementation has been suggested as a stratagem to reduce the severity of point-source Hg contamination, and it may also decrease the bioavailability and toxicity of atmospherically transported Hg (Rudd et al. 1980, 1983; Turner and Rudd 1983). The results of our experiments support these ideas.

Selenium can reduce bioaccumulation of Hg in several nonpredaceous fishes (Fig. 4B; Rudd et al. 1980; Turner and Rudd 1983). It seems probable that this effect would be increased further in organisms at higher positions within food webs; i.e. Se may interfere with the biomagnification of Hg. For example, lowered ^{203}Hg concentrations observed in perch (Fig. 4B, $P \sim 0.02$) appear to be further reduced in pike by the lowered assimilation of ^{203}Hg when accumulated from food sources (Fig. 5B, Se vs control). Additionally, the Se-induced reduction in the proportion of ^{203}Hg in skeletal muscle of pike (51% of control after 2 wk, Fig. 5A, food and water) may be an amplification of a similar decrease observed in pearl dace (75% of control) exposed for an equal length of time to Se at a concentration of 10 μg Se/L (unpublished data). This suggestion of interference with the transfer of Hg, depending on food web position, is further supported by observation of decreased accumulation of ^{203}Hg in predaceous aquatic insect larvae (*Sialis* and *Chaoborus*) living in ecosystems containing added Se (initially at 10 and 100 μg Se/L), without such decreases apparent in the detrivorous insects *Hexagenia* and *Chironomus* (B. E. Townsend, Freshwater Institute, Winnipeg, Man., personal communication). Therefore, the reduction observed in accumulation of ^{203}Hg in young suckers and perch, but not dace, after 2 mo at 1 μg

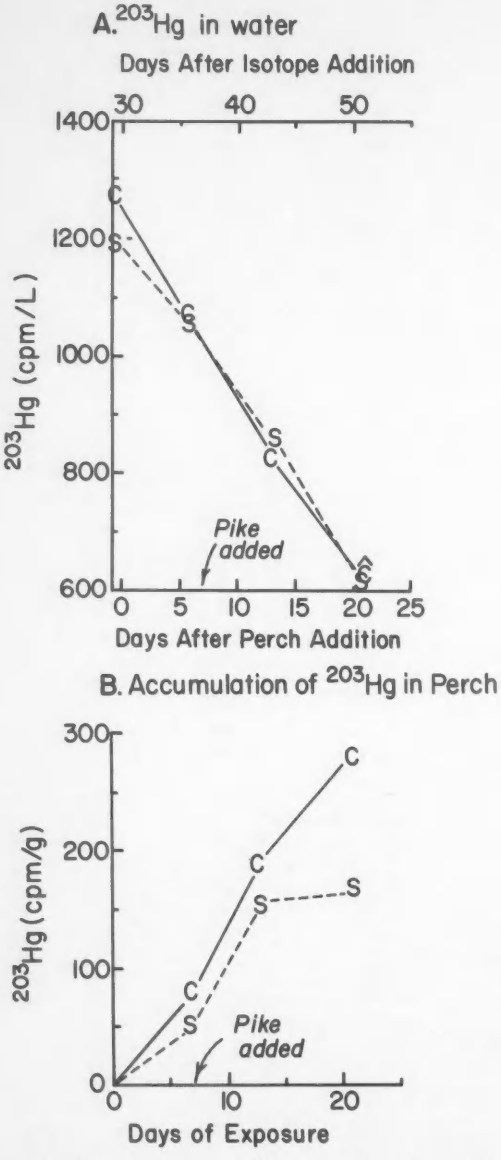


FIG. 4. (A) Concentrations of ^{203}Hg in the water column of the control (C) and Se (S) enclosures during the periods when perch and pike were exposed to ^{203}Hg. Concentrations shown for the last point are derived from model predictions (explained in Turner and Rudd 1983). (B) Mercury-203 in whole bodies of perch held in either the control (C) or Se (S) enclosures. Each point represents the antilog of the lognormal mean of three individuals. Concentrations of ^{203}Hg in perch of the Se and control conditions were significantly different ($P \sim 0.02$).

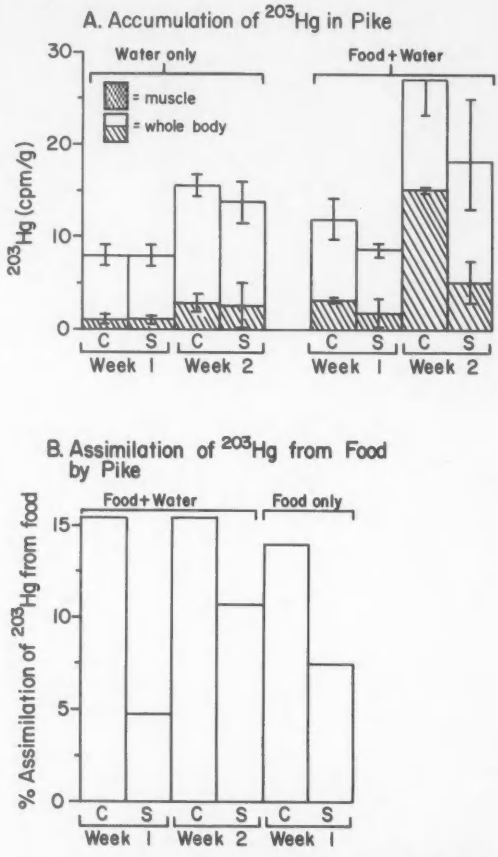


FIG. 5. (A) Concentrations of ^{203}Hg in northern pike held for 1 or 2 wk in the control (C) or added Se (S) enclosures. Pike were able to accumulate ^{203}Hg from food (perch) and water, or by uptake from water only. Concentration in skeletal muscle is shown in the hatched areas, while whole bodies are represented by the total height of each bar. Two standard error bars are shown about each mean of three individuals. (B) Efficiency of assimilation of ^{203}Hg from food (perch) by pike after 1 and 2 wk. To obtain the estimate of the burden of ^{203}Hg due to food alone, the uptake of ^{203}Hg from water, as measured in the water-only condition (Fig. 5A), was subtracted from body burdens observed in the food and water condition. In the corroborative, food-only experiment, the contribution of radioisotopes in food to the body burdens was observed directly.

Se/L (Turner and Rudd 1983) may be seen consistently in predaceous fish.

Although Se in water does not reduce bioaccumulation of Hg from water (Fig. 5A), levels of Hg do not have to be reduced either in the contaminated food or in the consumer for Se to exert its Hg-detoxifying effect (Chang et al. 1977; Friedman et al. 1978). The ability of Se to reduce Hg accumulation appears to be exerted via food intake (Fig. 5B).

TABLE 2. Ratios of tissue to whole body concentrations of ^{203}Hg (mean ± SD, $N = 3$.)

Tissue	Experimental condition	Week 1		Week 2	
		Water	Food + water	Water	Food + water
Gill	Control	9.49 (±1.49)	8.80 (±1.84)	9.07 (±1.68)	4.39 (±0.23)
	Se	9.96 (±1.67)	10.79 (±1.16)	5.19 (±3.01)	7.40 (±1.95)
Skin	Control	1.08 (±0.50)	1.26 (±0.34)	1.24 (±0.57)	0.64 (±0.10)
	Se	Not measured	0.59 (±0.83)[a]	0.76 (±0.37)	0.93 (±0.88)
Gut	Control	1.01 (±0.45)	3.36 (±0.78)	0.96 (±0.15)	3.30 (±0.49)
	Se	1.10 (±0.49)	2.56 (±0.45)	1.23 (±0.03)	2.03 (±0.61)
Liver	Control	7.95 (±2.21)	7.33 (±1.70)	9.78 (±2.21)	5.73 (±0.27)
	Se	7.84 (±1.12)	4.09 (±3.69)	15.33 (±5.26)	8.77 (±0.26)
Muscle	Control	0.10 (±0.05)	0.27 (±0.01)	0.18 (±0.06)	0.55 (±0.00)
	Se	0.14 (±0.12)	0.19 (±0.17)	0.20 (±0.18)	0.28 (±0.11)

[a] $N = 2$.

Furthermore, Se is transferred efficiently through food webs (Fig. 3B), so that elevated water concentrations would not have to be maintained continuously if periodic pulses could sufficiently load the food web. Any "addition" strategy should include monitoring levels in the food web to prevent buildup of unexpected, and potentially dangerous, concentrations of Se.

Our work suggests that increased emphasis should be placed on the potential toxicity to fish of elevated Se concentrations in food. Moreover, before Se can be used as an aquatic ameliorating agent with some degree of confidence, we should assess further both what minimum ratio of Se:Hg is effective in reducing assimilation of Hg and whether there is any time dependency of this effect.

Acknowledgments

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The English–Wabigoon River System: V. Mercury and Selenium Bioaccumulation as a Function of Aquatic Primary Productivity

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RUDD, J. W. M., and M. A. TURNER. 1983. The English–Wabigoon River system: V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. 40: 2251–2259.

An experiment was carried out in four 100-m^3 in situ enclosures to determine the effect of primary production rate on mercury and selenium concentrations of biota and to test the possibility of ameliorating mercury pollution problems by increasing ecosystem primary productivity. Two enclosures were controls. Primary productivity in a third enclosure was increased fourfold by addition of $NaNO_3$ and NaH_2PO_4. This stimulation was not sufficient to change pH although the growth rate of fish was enhanced. In this enclosure, mercury concentrations in pearl dace (*Semotilus margarita*) whole body and muscle samples increased two- and five-fold, respectively, exceeding the dilution of mercury by the enhanced growth rates. In the fourth enclosure, at the highest rate of nutrient addition, primary productivity was increased ninefold, pH was elevated from about 7.9 to 9.2, and the growth rate of fish was stimulated. In this case, the elevation of pH retarded the rate of mercury bioaccumulation in comparison with the enclosure of intermediate productivity. Based on these results, stimulation of primary productivity is not recommended as a mercury ameliorating procedure. Stimulation of primary productivity resulted in a general decrease in the concentration of selenium in the aquatic food chain probably resulting from dilution of selenium by enhanced growth rates of fish and other biota.

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Une expérience a été menée dans quatre enceintes de 100 m^3 in situ dans le but de déterminer les effets du taux de production primaire sur les concentrations de mercure et de sélénium des biocénoses et vérifier la possibilité d'atténuer les problèmes de contamination par le mercure en augmentant la productivité primaire de l'écosystème. Deux enceintes ont servi de témoins. Dans une troisième, la productivité primaire a été augmentée de quatre fois par addition de $NaNO_3$ et de NaH_2PO_4. Ce traitement n'a toutefois pas suffi à modifier le pH, bien que le taux de croissance des poissons ait augmenté. Dans cette enceinte, les concentrations de mercure dans des échantillons du corps entier et du muscle de mulets perlés (*Semotilus margarita*) ont augmenté de deux à cinq fois respectivement, dépassant la dilution du mercure par suite de taux de croissance accélérés. Dans la quatrième enceinte, qui reçut la plus importante addition de nutriments, la productivité primaire a augmenté de neuf fois, le pH a passé de 7,9 à 9,2 et le taux de croissance des poissons a été stimulé. Dans ce cas, l'élévation du pH a retardé le taux de bioaccumulation du mercure par rapport à l'enceinte de productivité intermédiaire. Sur la base de ces résultats, on ne peut recommander la stimulation de la productivité primaire comme contre-mesure de contamination par le mercure. La stimulation de la productivité primaire cause une diminution générale de la concentration du sélénium dans la chaîne alimentaire aquatique, probablement par suite de la dilution du sélénium par des taux de croissance accélérés des poissons et autres biocénoses.

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FACTORS controlling the concentration of Hg in fish and other aquatic biota are only partially understood. Improved knowledge of these factors could be useful in designing methods to ameliorate Hg contamination problems. One of these controlling factors is primary productivity. Stimulation of primary productivity may affect the Hg concentration of fish in two opposing ways. First, an increased supply of decomposable algal carbon could stimulate Hg methylation by microorganisms, which would tend to increase the Hg concentration of fish (Furutani and Rudd 1980; Rudd et al. 1983). Second, stimulation of primary production could tend to reduce methyl mercury concentrations in fish, if the

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TABLE 1. Chronology of enclosure additions.

Days after isotope addition	Addition
−12 to −10	Enclosures filled
−10	3 kg NaCl per enclosure. Low-nutrient enclosure: 7.12 g NaH_2PO_4 and 127 g $NaNO_3$ High-nutrient enclosure: 18.04 g NaH_2PO_4 and 314 g $NaNO_3$
−5	Begin weekly additions. Low nutrient: 3.6 g NaH_2PO_4 and 63 g $NaNO_3$ High nutrient: 9.02 g NaH_2PO_4 and 157 g $NaNO_3$
0	$^{203}Hg(NO_3)_2$ and $H_2{}^{75}SeO_3$ to each enclosure on June 4, 1979
4	60 pearl dace per enclosure at 40 kg/ha (~5 g each)
7	18 crayfish per enclosure (~7 g each)
9	6 clams per enclosure (~120 g each)
30	Termination of experiment

bioaccumulated Hg is diluted by faster growth rates of the fish. Also, increases in lake water pH, resulting from higher primary productivity, might promote the formation of dimethyl mercury, which is more volatile than monomethyl mercury. Consequently, Hg may be lost to the atmosphere rather than be bioaccumulated (Fagerstrom and Jernelov 1972; Jernelov et al. 1975, 1976; Beijer and Jernelov 1979).

During 1979, we carried out an experiment designed to test the effect of primary production rate on Hg concentrations of fish and other biota. The experiment was done in large enclosures situated in Clay Lake (50°03′N, 93°30′W), which is part of the Hg-polluted English–Wabigoon River system of northwestern Ontario (Scott and Armstrong 1972; Armstrong and Hamilton 1973; Scott 1974; Armstrong and Scott 1979). Concentrations of Hg in fish and other members of the food chain in control enclosures were compared with concentrations of Hg in the same organisms maintained in enclosures that had received additions of nitrogen and phosphorus to stimulate primary productivity.

The effect of primary production rate on Se bioaccumulation was also tested because of the potential role of Se as a Hg ameliorating agent (Rudd et al. 1980; Turner and Rudd 1983; Turner and Swick 1983).

Methods

The experiments were conducted in four 10-m-diameter, 1.5-m-deep, cross-laminated polyethylene enclosures with water-tight bottoms. The installation of the enclosures is described in Rudd and Turner (1983).

Enclosure Additions

Each enclosure received additions of ^{203}Hg, ^{75}Se, and NaCl as described in Rudd and Turner (1983). NaCl was added to monitor leakage of the enclosures, which was undetectable during the experiment.

Two of the enclosures were controls and received no other chemical additions. The other two enclosures (low and high nutrient) were fertilized periodically with NaH_2PO_4 and $NaNO_3$ (reagent grade) (Table 1). Fertilization commenced 10 d before day 0, the day of radioisotope additions. Fertilization rates were highest before day 0 to allow time for the enhanced primary productivity to stimulate microbial activity and growth of biota before addition of the Hg and Se radioisotopes.

Aside from plankton added during filling of the enclosures no organisms were present at the beginning of the experiment. Pearl dace (*Semotilus margarita*) and crayfish (*Orconectes virilis*) were put into the enclosures on days 4 and 7, respectively (Table 1).

Radioisotope Sampling and Analyses

Water samples were analyzed for ^{203}Hg and ^{75}Se speciation (particulate, ionic, organic, and unassociated) as described in Rudd and Turner (1983).

Bioaccumulation of radioisotopes was monitored in clams (*Anodonta* sp.) and crayfish caged individually in epoxy-coated wire mesh cages and in free-swimming pearl dace. Analytical methods are described in Rudd and Turner (1983).

Usually, concentrations of radioisotopes in fish were compared between the fertilized and control enclosures. On occasion, to account for dilution of radioisotope by enhanced growth rates, the "body burdens" of the radioisotopes have been presented. Body burden (cpm per fish) is the total quantity of radioisotope in a fish. It was calculated by multiplying the concentration (cpm per gram) of a radioisotope by the mass (grams) of the fish.

Concentrations of $CH_3{}^{203}Hg^+$ in muscle samples of pearl dace were determined by organic solvent extraction of the $CH_3{}^{203}Hg^+$ from the samples, followed by scintillation counting as described in Turner and Rudd (1983). Bioaccumulation of ^{203}Hg and ^{75}Se by periphyton and zooplankton was followed as described in Rudd and Turner (1983).

Other Analyses

Primary productivity samples were collected using a rigid PVC tube, which uniformly sampled the entire depth of the water column. Productivity was assayed by the ^{14}C-incubator technique of Fee (1973a) and Shearer (1976a, 1976b). Daily estimates of primary productivity for cloudless conditions were made using an empirical model (Fee 1973b). These rates are probably slight overestimates of actual rates because of natural cloud conditions (Fee 1980). The ^{14}C method may overestimate net primary production (Steeman Nielsen and

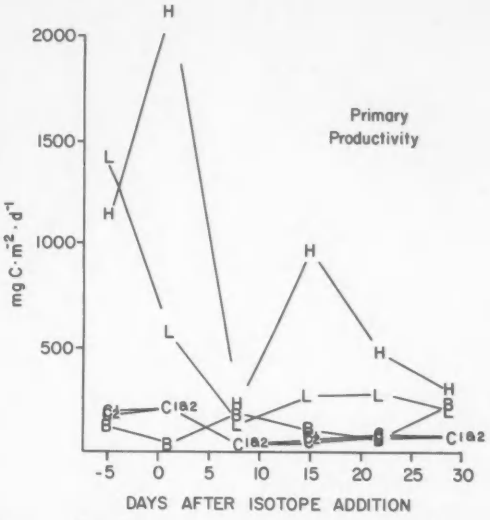

FIG. 1. Rates of primary production under cloudless conditions in the control (C_1 and C_2), low-nutrient (L), and high-nutrient enclosures (H) and in Clay Lake outside the enclosures (B).

Hansen 1959; Turner 1981). However, this should not affect the relative amounts of decomposable algal material produced in the control and nutrient enclosures, which is the basis of interpretation for this experiment.

Water samples from the enclosures and lake were analyzed biweekly for particulate and dissolved carbon, nitrogen, and phosphorus fractions, sulfate, pH, chloride, total suspended solids, and soluble reactive silica using methods described in Stainton et al. (1977).

Results

PRIMARY PRODUCTIVITY

Rates of primary productivity in the two control enclosures were very similar (Fig. 1), indicating that enclosure-to-enclosure variability was not an important factor in the interpretation of these results. Primary productivity in the controls and in the lake was also similar. Fertilization caused a large increase in the quantity of carbon fixed in the nutrient enclosures. During the 34-d period that primary productivity was measured (day 5−day 29), the total quantities of carbon fixed in the high- and low-nutrient enclosures exceeded the controls by 9 and 4 times, respectively.

As a result of stimulating primary productivity, the pH in the high-nutrient enclosure increased (Fig. 2). During the experiment the mean pH in the high-nutrient enclosure was 9.2 ± 0.2 (SD, $N = 4$). The other enclosures averaged 7.9 ± 0.3 ($N = 12$).

Dissolved and suspended phosphorus, nitrogen, and carbon concentrations were generally about twofold higher in the nutrient-enriched enclosures than in the control enclosures and the lake (Table 2). Nitrate concentration was lowest in the unfertilized control enclosures. This was likely due to isolation of the control enclosures from nitrogen inputs from the sediments. These differences in nitrate concentration did not, however, lower primary productivity rates as compared with the lake (Fig. 1).

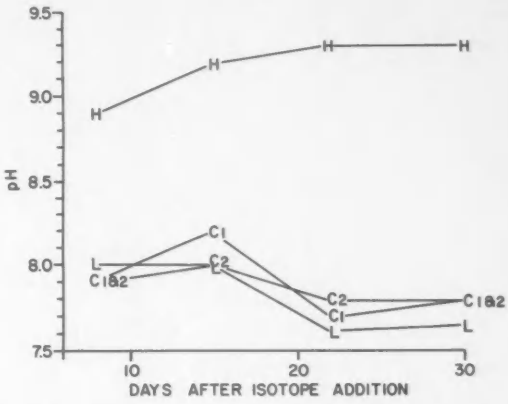

FIG. 2. pH of water samples taken from the high-nutrient (H), low-nutrient (L), and control (C_1 and C_2) enclosures.

DISSOLVED AND SUSPENDED ^{203}Hg AND ^{75}Se

Increasing primary productivity had no apparent effect on the rate of loss of ^{203}Hg from the water columns of the enclosures (Fig. 3). Stimulating the productivity increased the percentage of ^{203}Hg associated with particulate material from a mean of 27.0 ± 0.5% in the controls to 37.4 ± 0.9% in the nutrient enclosures (Table 3). This means that in both nutrient enclosures about 40% more ^{203}Hg was available to the food chain in the particulate fraction. Most of this increase in ^{203}Hg-particulate material appears to have been derived from the unassociated fraction with a lesser amount contributed by the ionic fraction. Although 40% more ^{203}Hg was in the particulate fraction, the specific activity per unit carbon of this material (cpm per gram) was lower in the nutrient enclosures because particulate carbon concentrations had doubled following fertilization (Table 2).

There was no apparent effect on the loss of ^{75}Se from the water column due to nutrient addition (Fig. 3). An average of 20% of the ^{75}Se was removed from the water column of all enclosures during the experiment. Stimulating the primary productivity also had no discernable effect on partitioning of the ^{75}Se remaining in the water column (Table 3). As in previous research (Rudd et al. 1980; Turner and Rudd 1983), more than 50% of the ^{75}Se in all of the enclosures was associated with the charcoal (organic) fraction.

BIOACCUMULATION OF ^{203}Hg AND ^{75}Se

Mercury and Se bioaccumulation by pearl dace, crayfish, and zooplankton was very similar between the two control enclosures (Fig. 4). The range of control mean values usually

TABLE 2. Water chemistry in the control and nutrient addition enclosures and in Clay Lake outside the enclosures on day 20 of the experiment (NS = no sample).

μg/L	Control 1	Control 2	Low nutrient	High nutrient	Lake
NH_4^+	50	50	50	60	60
NO_2^-	3	2	3	6	5
NO_3^-	12	18	67	155	77
TDN	520	490	640	860	660
Susp N	105	100	186	194	89
TDP	15	15	20	27	30
Susp P	9	9	23	30	12
DOC	1440	1700	1650	1690	1890
Susp C	560	510	1080	1160	570
SRSi (mg/L)	0.48	0.41	0.06	0.14	0.81
SO_4^{2-} (mg/L)	7.4	7.8	7.8	8.4	NS
TSS (mg/L)	2	2	3	4	NS

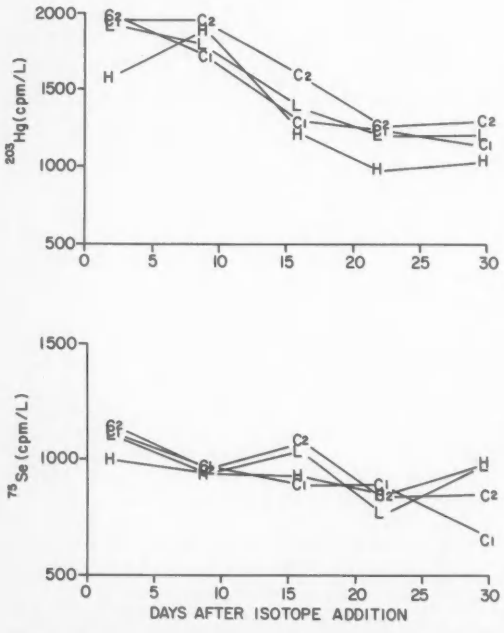

FIG. 3. Concentrations of ^{203}Hg and ^{75}Se in the water columns of the control (C_1 and C_2), low-nutrient (L), and high-nutrient (H) enclosures as a function of time. Isotope concentrations are the sums of all of the Hg and Se species.

was less than 10% of the combined means for all three types of organisms. This indicates that enclosure-to-enclosure variability was not an important factor in interpreting the ^{203}Hg and ^{75}Se bioaccumulation results. Trends of bioaccumulation were very similar for both Hg and Se radioisotopes. Pearl dace whole body concentrations plateaued after 21 d of exposure. Zooplankton peaked in concentration after 9 d and then declined progressively for the remainder of the experiment. Whole body ^{203}Hg and ^{75}Se concentrations of crayfish increased steadily over the 23 d of exposure.

The dry weight of periphyton peaked on day 22 in all of the enclosures at 80 and 50 mg/cm^2 in the high- and low-nutrient enclosures, respectively, and at 20 mg/cm^2 in the controls. On day 22, ^{203}Hg concentrations per unit dry weight in the high- and low-nutrient enclosures were reduced to 50 and 63%, respectively, of the combined controls. Concentrations of ^{75}Se were reduced to a smaller extent. In both the high- and low-nutrient enclosures, ^{75}Se concentrations may have been reduced to about 80% of the control enclosures.

Stimulation of primary productivity increased zooplankton standing crop and hence probably stimulated zooplankton growth. While biomass in the control enclosures remained stable throughout the experiment, the dry weight of zooplankton in the nutrient enclosures peaked at 6 times above the controls between day 2 and 16. Despite the larger biomass of zooplankton in the fertilized enclosures, ^{203}Hg concentrations were similar at the end of the experiment in all enclosures (Fig. 5). Concentration of ^{75}Se in zooplankton in the nutrient enclosures was consistently half of the control values (Fig. 5).

After 13 d of exposure, concentrations of ^{203}Hg and ^{75}Se in the foot muscle of clams were significantly lower in the nutrient enclosures than in the controls, with ^{75}Se being reduced to a greater extent than ^{203}Hg (Table 4). Concentrations of both isotopes were lower in the high- than in the low-nutrient enclosure, suggesting a dose-dependent response to nutrient addition. Growth of the clams during the exposure period was not determined, but in view of the faster growth observed in the other organisms, reduced isotope concentrations were probably a consequence of enhanced growth rates.

The ^{203}Hg concentrations of crayfish were consistently higher in the low-nutrient enclosure than in the controls, whereas ^{203}Hg concentrations in the high-nutrient enclosure were not consistently elevated (Fig. 6). Bioaccumulation of ^{75}Se in crayfish was clearly enhanced in the nutrient enclosures, particularly after 3 wk of exposure (Fig. 6).

In response to enhanced primary productivity, pearl dace in

TABLE 3. Partitioning of ^{203}Hg and ^{75}Se in five water samples taken from control and nutrient enclosures during a 30-d experiment. For sampling dates see Fig. 3. Data represent means ± SD.

Enclosure	% particulate	% charcoal	% ionic	% unassociated
		^{203}Hg		
Control I	27.4 (±8.1)	24.9 (±8.3)	14.5 (±5.3)	33.1 (±15.3)
Control II	26.5 (±4.7)	24.6 (±7.5)	13.4 (±4.8)	35.4 (±11.3)
Low nutrient	36.0 (±10.0)	23.3 (±4.9)	12.2 (±4.7)	28.5 (±10.4)
High nutrient	38.8 (±5.5)	26.0 (±5.6)	11.9 (±2.0)	23.3 (±5.5)
		^{75}Se		
Control I	23.2 (±16.9)	51.1 (±12.3)	25.7 (±11.5)	—
Control II	25.0 (±19.6)	53.4 (±16.9)	21.6 (±9.8)	—
Low nutrient	23.6 (±17.1)	53.3 (±13.2)	23.1 (±8.5)	—
High nutrient	23.9 (±19.0)	55.0 (±14.4)	21.1 (±7.5)	—

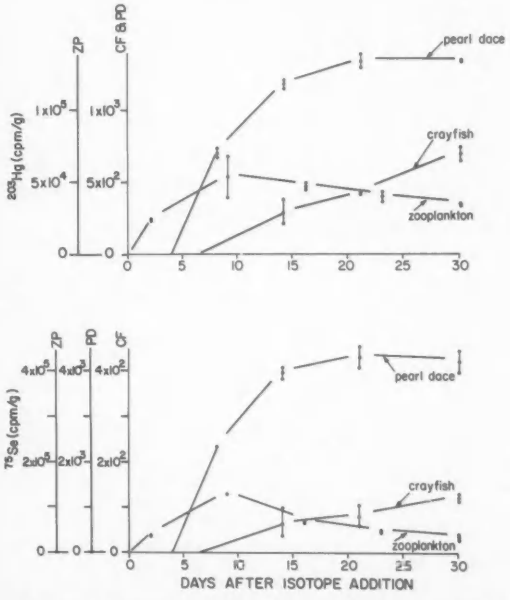

FIG. 4. Time course estimates of ^{203}Hg and ^{75}Se concentrations in pearl dace (PD) and crayfish (CF) whole bodies and in zooplankton (ZP) samples taken from the control enclosures. Vertical lines connect the range of the lognormal means of concentrations in the two control enclosures.

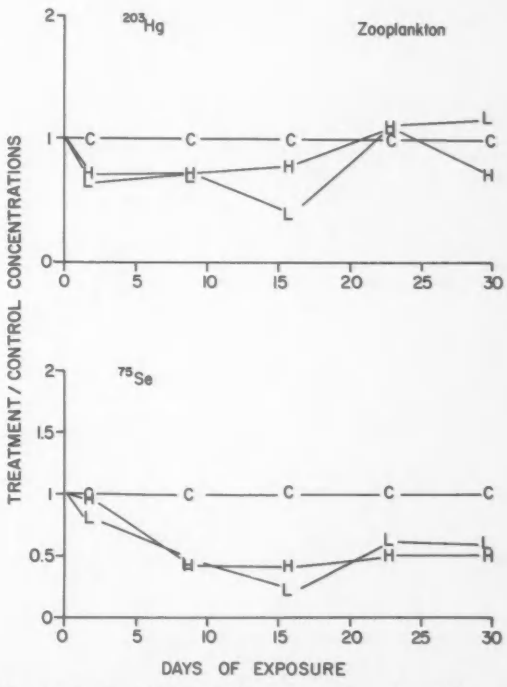

FIG. 5. Ratios of ^{203}Hg and ^{75}Se concentrations of zooplankton samples taken from the high-nutrient (H) and low-nutrient (L) enclosures and from the controls (C). Ratios were calculated by dividing the concentrations of samples from the nutrient enclosures by the average concentration of samples from both control enclosures.

the high-nutrient enclosure grew faster than in the low-nutrient enclosure ($P < 0.1$, paired t-test) and much faster than in the control enclosures ($P < 0.05$) (Fig. 7A). Until the last sampling date there was no marked difference in body burdens of ^{203}Hg (cpm per fish) between dace in the controls and nutrient enclosures. However, after 27 d of exposure, body burdens in the high- and low-nutrient enclosures were 1.5 and 3 times those of the controls, respectively (Fig. 7B). In terms of ^{203}Hg concentrations (cpm per gram) (Fig. 7C), pearl dace in the high-nutrient enclosure were not different from the controls on the last sampling day, but dace in the low-nutrient enclosure had twice the ^{203}Hg concentration of the controls. These trends were accentuated in pearl dace muscle samples (Fig. 7D). On the last sampling day, ^{203}Hg concentrations in muscle were 4.8 and 2.7 times higher in the low- and high-nutrient enclosures than in the control

TABLE 4. Concentrations of ^{203}Hg and ^{75}Se in the foot muscle of clams held for 13 d in enclosures of varying primary productivity. One standard deviation is obtained by multiplying or dividing the lognormal mean by the factor given in parentheses.

Treatment	n	^{203}Hg (cpm/g $\overset{\times}{\div}$ SD)	^{203}Hg (% of controls)	^{75}Se (cpm/g $\overset{\times}{\div}$ SD)	^{75}Se (% of controls)
Combined controls	10	265 ($\overset{\times}{\div}$1.28)	100	190 ($\overset{\times}{\div}$1.48)	100
Low nutrient	5	192 ($\overset{\times}{\div}$1.21)	73	92 ($\overset{\times}{\div}$1.30)	48
High nutrient	5	141($\overset{\times}{\div}$1.55)	53	64 ($\overset{\times}{\div}$1.45)	34

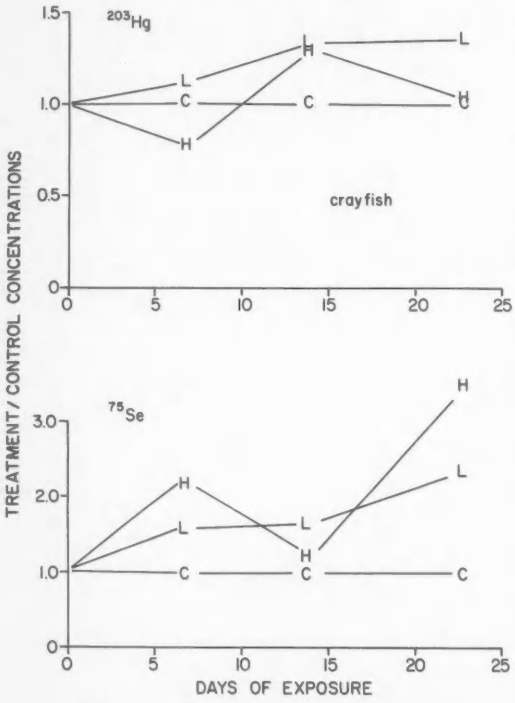

FIG. 6. Ratios of ^{203}Hg and ^{75}Se concentrations in whole body crayfish samples taken from the high-nutrient (H) and low-nutrient (L) enclosures and from the controls (C). Ratios were calculated by dividing concentrations of samples from the nutrient enclosures by the average concentration of samples from both control enclosures.

enclosures, respectively. These large increases in ^{203}Hg on the last sampling date were verified by the analyses of four additional pearl dace muscle samples from each enclosure.

The ^{75}Se body burdens (cpm per fish) of pearl dace in the nutrient enclosures were reduced to about 75% of the controls (Fig. 7B). Whole body ^{75}Se concentrations (cpm per gram) were reduced to about 60% of those in the controls, independent of nutrient loading (Fig. 7C). Concentrations of ^{75}Se in pearl dace muscle samples were reduced by approximately the same amount as the whole body concentrations (Fig. 7D).

Discussion

EFFECTS OF PRIMARY PRODUCTIVITY ON MERCURY BIOACCUMULATION

The four- and nine-fold stimulation of primary productivity in the nutrient enclosures appeared to affect two factors known to influence fish Hg concentration. First, the growth rate of the fish responded directly to the level of fertilization. Pearl dace in the high-nutrient enclosure grew significantly faster than dace in the low-nutrient enclosure and much faster than in the control enclosures (Fig. 7A). This would tend to reduce the Hg concentration of the fish by diluting the bioaccumulated Hg. Second, an increased rate of supply of algal carbon to the bacterial decomposers apparently stimulated rates of methyl mercury production. Higher rates of Hg methylation would tend to increase the Hg concentration of the fish. Large increases (>30-fold) in methylation rates and the Hg concentration of fish have been observed in other experiments when decomposable carbon was added directly to enclosures at Southern Indian Lake and to Clay Lake water and sediments (Furutani and Rudd 1980; Rudd et al. 1983). In our experiment, ^{203}Hg body burdens (cpm per fish) increased rapidly in the nutrient-enriched enclosures (Fig. 7B). This trend was even more evident in the dace muscle samples, which exhibited large increases in ^{203}Hg concentration on the last sampling date (Fig. 7D). The ^{203}Hg in the muscle samples was 77 ± 8 and 83 ± 3% $CH_3{}^{203}Hg^+$ in the low- and high-nutrient enclosures, respectively, but only 60 ± 3% $CH_3{}^{203}Hg^+$ in the control enclosures. The much lower concentration of $CH_3{}^{203}Hg^+$ and the lower percentage of $CH_3{}^{203}Hg^+$ in dace from the control enclosures suggest a lower Hg methylation rate in these unfertilized enclosures.

The delay in elevation of fish and crayfish Hg concentrations in the nutrient enclosures likely resulted from the time required for the increased primary productivity to affect the decomposition side of the food chain and the time required for the methylated mercury to move up the food chain. The delay was not due simply to changes in the growth rate of the fish towards the end of the experiment because rates of growth were constant in each enclosure throughout the experiment (Fig. 7A).

Even though there was more than twice as much algal

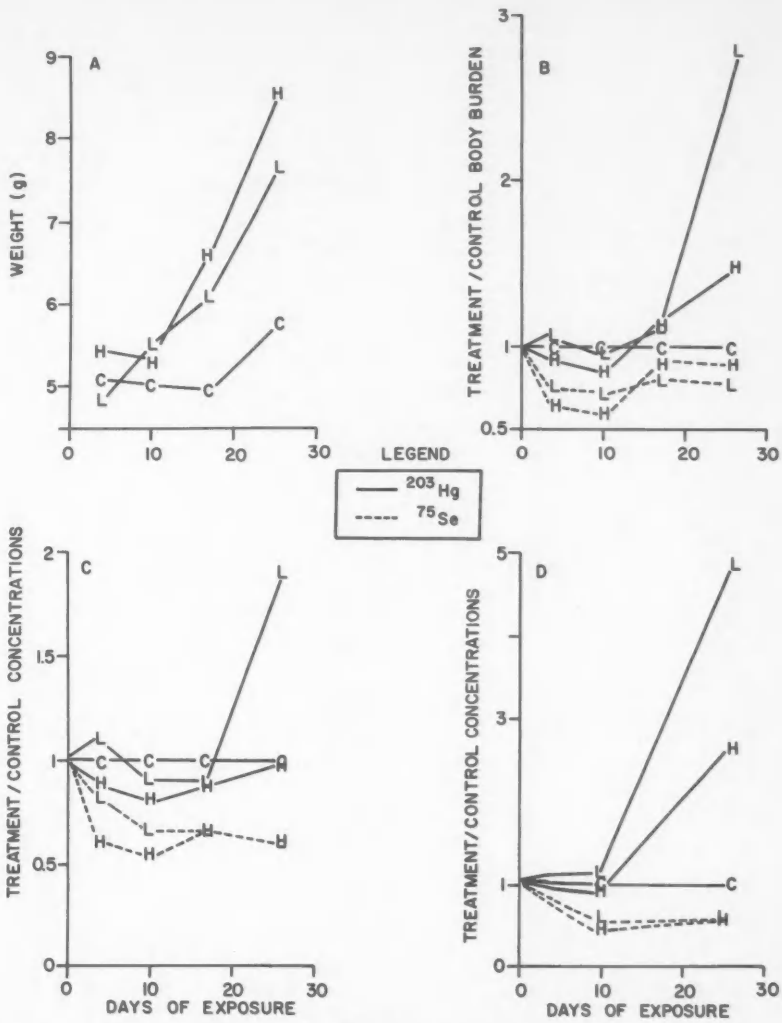

FIG. 7. (A) Lognormal mean weights of pearl dace taken from the controls (C), high-nutrient (H), and low-nutrient (L) enclosures. (B) Ratios of ^{203}Hg and ^{75}Se body burdens (cpm per fish) of pearl dace taken from the high-nutrient (H) and low-nutrient (L) enclosures relative to the controls (C). Ratios were calculated by dividing concentrations of samples from the nutrient enclosures by concentrations of samples from the control enclosures. Solid and broken lines are ^{203}Hg and ^{75}Se, respectively. (C) Ratios of ^{203}Hg and ^{75}Se concentrations in pearl dace whole bodies from the high-nutrient (H) and low-nutrient (L) enclosures relative to the controls (C). Ratios were calculated by dividing concentrations of samples from the nutrient enclosures by concentrations of samples from the control enclosures. Solid and broken lines are ^{203}Hg and ^{75}Se, respectively. (D) Ratios of ^{203}Hg and ^{75}Se concentrations in pearl dace skeletal muscle samples from the high-nutrient (H) and low-nutrient (L) enclosures relative to the control enclosures (C). Ratios were calculated by dividing concentrations of samples from the nutrient enclosures by concentrations of samples from the control enclosures. Solid and broken lines are ^{203}Hg and ^{75}Se, respectively.

carbon (bacterial food) in the high-nutrient enclosure as compared with the low-nutrient enclosure, the body burdens of Hg in fish and concentrations of Hg in the crayfish were lower in the high-nutrient than in the low-nutrient enclosure (Fig. 6,

7C, and 7D). A possible explanation is the 40-fold difference in hydrogen ion concentration between the enclosures, resulting from the higher photosynthetic rates in the high-nutrient enclosure. An increase in pH may decrease the efficiency with which fish bioaccumulate Hg (Drummond et al. 1974). Moreover, high pH may also favor the formation of dimethyl mercury (Fagerstrom and Jernelov 1972). Dimethyl mercury has less affinity for protein and is more volatile than monomethyl mercury; thus, it may be lost to the atmosphere rather than bioaccumulated (Jernelov et al. 1975, 1976).

Although elevated pH appears to have inhibited Hg bioaccumulation somewhat as predicted by other researchers (Jernelov et al. 1975, 1976; Beijer and Jernelov 1979), increasing productivity, pH, and the growth rate of fish did not cause decreases in the Hg concentration of the fish as compared with the controls. This was probably because there was an increase in Hg methylating activity at high pH, which was suffcient to overcome the effect of dilution by the increased growth rate of the fish.

The overall effects of increasing primary productivity on Hg concentration of fish appear to be a complex interrelationship between stimulation of the growth rate of fish and microbial Hg methylation rate and, in some cases, a change in pH, which may reduce either bioaccumulation efficiency of CH_3Hg^+ by fish or change the form of methylated mercury produced by microorganisms. Increases in primary productivity that were not large enough to affect ecosystem pH produced the largest increases in Hg concentration of pearl dace and crayfish. These conditions appear to be optimal for Hg methylation and bioaccumulation.

The periphyton and the clams were the only members of the food chain that consistently decreased in Hg concentration as a result of stimulating primary productivity. This may have been caused by faster growth rates in the nutrient enclosures (2.4−4.0 times for periphyton) together with their known low rates of Hg bioaccumulation as compared with higher members of the food chain (Smith et al. 1975; Huckabee et al. 1979). Mercury bioaccumulation by zooplankton and pearl dace eventually either kept pace with or exceeded dilution due to enhanced rates of growth (Fig. 5, 7A−7D). A similar trend was also observed in crayfish although growth could not be estimated in this case (Fig. 6).

Increasing primary productivity by addition of algal nutrients has been suggested as a technique for reducing Hg concentrations of fish (e.g. Beijer and Jernelov 1979). Our experimental results suggest that this could be unsuccessful. Instead, this treatment would probably result in stable or increased Hg concentrations in fish, depending on the extent of pH changes following nutrient addition. Our results suggest that lowering primary productivity by reducing nutrient input would lower methylation rates, where primary production is the main source of bacterial substrate. However, this treatment would probably also reduce fish growth rates; thus, the long-term effect on fish Hg concentrations is at present difficult to predict.

Effects of Primary Productivity on Selenium Concentrations

The effects of primary production rate on Se bioaccumulation are of interest because Se can be toxic in excessive quantity (Klaverkamp et al. 1983; Turner and Rudd 1983) and because the Se concentration of food chain organisms and fish may be an important factor in determining the efficacy of Se as an ameliorating agent for Hg-contaminated waterways (Rudd et al. 1980; Turner and Rudd 1983; Turner and Swick 1983).

Stimulating primary productivity by nutrient addition resulted in a decrease of about 50% in the ^{75}Se concentration of the periphyton, zooplankton, clams, and pearl dace in the nutrient enclosures relative to the controls (Fig. 5 and 7C; Table 4). This was probably due to dilution of ^{75}Se by enhanced growth rates of the biota in the nutrient enclosures. The only exception was the crayfish, which had higher ^{75}Se concentrations in the nutrient enclosures than in the controls. The crayfish also had much lower ^{75}Se concentrations (cpm per gram) than did the zooplankton or pearl dace (Fig. 4). This was probably because the crayfish were caged and fed white sucker (*Catostomus commersoni*) flesh that was not labeled with ^{75}Se. At the trace Se levels present in this experiment (<0.2 μg Se/L), Se is predominantly bioaccumulated through the food chain (Turner and Swick 1983). Thus, different rates of accumulation between crayfish in control and nutrient enclosures probably reflect an effect on bioaccumulation of Se from water, which is usually a minor source.

If nutrient addition were used as a Hg amelioration procedure, our experiment suggests that stimulation of primary productivity would increase the ratio of Hg:Se in fish. This would increase the toxicity of Hg to the fish consumer (Friedman et al. 1978). The ratio is already high in the English−Wabigoon system because of Hg contamination and because Se concentrations of fish are low when compared with other bodies of water (Turner and Rudd 1983). We suggest that there are at least three reasons why the Hg:Se ratio would increase: (1) there could be an increase in the concentration of Hg in fish at higher levels of primary productivity, (2) concentrations of Se may be reduced by the enhanced growth rates of the fish, and (3) the efficiency of Hg bioaccumulation by fish would probably be enhanced because the Se concentration of food chain organisms is inversely related to the efficiency with which fish accumulate Hg (Turner and Swick 1983). This enclosure experiment also suggests to us that if the rate of primary production in a productive aquatic system were reduced, there would be a lowering (improvement) of the Hg:Se ratio in fish.

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Comment on Evidence of Acidification on Some Nova Scotia Rivers and Its Impact on Atlantic Salmon, *Salmo salar*

COMMENT

We cannot agree with the interpretation of the SO_4^{2-} data given in Tables 4 and 5 by Watt et al. (1983). We believe that the increased input of acid (mostly sulfuric) apparent in Table 4 is at least partly the result of differences in methodology employed in the two data sets (1954–55 vs 1980–81). We cannot accept the statement that "the analytical techniques (Thomas 1959) are all comparable with the 1980–81 data, except possibly for aluminum." Thomas employed a turbidimetric sulfate method (Thomas 1959, 1960), while the 1980–81 sulfate data were obtained with methylthymol blue (MTB) colorimetry (Anonymous 1979). The latter is more susceptible to color interferences than the former, which is an important consideration, particularly in the highly colored rivers such as Roseway given in Tables 4 and 5.

Based on our initial work in the Mersey River drainage system we also reported high SO_4^{2-} concentrations obtained with the MTB method in highly colored, humic waters (Kerekes 1980; Kerekes et al. 1982). Since then we have come to realize that the MTB method gives higher SO_4^{2-} values in colored waters compared with that from the ion chromatography (IC) (Anonymous 1983) method, which is free from color interferences (Kerekes 1981). We have concluded that the MTB method gives consistently higher SO_4^{2-} values than that of the IC method with a discrepancy between the two methods increasing with an increase in water color (Howell and Kerekes 1983; Pollock 1983). By contrast, the turbidimetric method employed by Thomas (1959, 1960) is color corrected and the SO_4^{2-} determinations are similar to that obtained with the IC method.

We found that in the Mersey River at Mill Falls, a site farther upstream from that sampled by Watt et al. (1983), the average SO_4^{2-} concentrations (mean of five samples between December 18, 1981, and June 2, 1982; color = 80 Hazen units) were 72.4 μeq/L measured with the MTB method and 49.1 μeq/L measured with the IC method. The concentrations corrected for sea salts were 59.3 and 36.0 μeq/L, respectively. The markedly lower SO_4^{2-} values obtained by IC give charge balances where the sum of cations are greatly overabundant relative to the sum of anions. However, Oliver (1981) and Oliver et al. (1983) indicated that humic (fulvic) substances contribute to the acidity of colored natural waters. They showed that where the organic carbon is humic material and the dissolved organic carbon concentration is known, the organic anion (A^-) concentration that results from the dissociation of humic material can be estimated. In the Mersey River example, given above, the estimated A^- is 79.2 μeq/L, which makes up 31.0% of the sum of anions, while in more colored rivers (120 Hazen units), A^- may constitute about 50% of the anions (Kerekes and Howell 1983). The inclusion of the A^- concentration in the sum of anions, along with the IC SO_4^{2-} concentration, gives a good charge balance with a balance error <5%.

We suggest that in humic, colored waters such as the Roseway, Medway, Mersey, and LaHave rivers, the MTB SO_4^{2-} determination gives biased results, and water chemistry, as it relates to acidity, cannot be considered without an adequate treatment of the organic anions present that contribute to the acidity of these waters. Given the SO_4^{2-} data presented by Watt et al. (1983) and not knowing the A^- present, one cannot estimate what percentage of the quantities of acidity change (1954–55 vs 1980–81) is attributable to SO_4^{2-} concentrations in the four rivers presented —

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REPLY

The sulfate data are of relatively minor importance in our paper inasmuch as they affect neither of the main conclusions, viz that some Nova Scotian rivers have become acidified since the 1950's and that Atlantic salmon (*Salmo salar*) stocks have declined in apparent response to this acidification. We are aware, however, that the overall issue of the contribution of SO_4^{2-} to acidity has developed political significance.

We were acquainted with Cronan's (1979) results demonstrating a direct relationship between SO_4^{2-} measured by the automated methylthymol blue technique and color from an "aqueous extract of balsam fir litter." Cronan found interference at color levels >200 Hazen units, and he indicated that the threshold for interference lies between 100 and 200 units. His results would indicate that our data from the Medway, Mersey, LaHave, and Musquodoboit rivers are well below the level of significant interference; and even with Roseway River data, only the most highly colored samples would be borderline. Statistical analyses indicate that colors in all five rivers were significantly lower ($P < 0.01$) in the 1980–81 data than in Thomas' (1960) data from 1954–55. We could find no significant positive correlation between color and SO_4^{2-}; Musquodoboit, however, shows a significant negative correlation. Given the lack of a positive correlation in the data and the relatively high color threshold suggested by Cronan (1979), we concluded that our sulfate data would not be significantly affected by this potential source of interference.

Kerekes and Pollock have now provided evidence for significant interference at 80 Hazen units. This color level is higher than any from our Medway, Mersey, or LaHave data, which average 39, 34, and 35 units, respectively, but it is lower than most samples from the Roseway site. We concede that our reported SO_4^{2-} levels for the Roseway are probably too high.

T. Pollock's (personal communication) analytical data indicate that no significant interference occurs at color levels <50 units and that color interference is higher in headwater streams (near humate sources) than on the lower main stems of the rivers. All of our sampling stations were on the main stems near the mouths. Only one sample (70 units) from the Medway and one sample (60 units) from the Mersey (none from LaHave) exceeded 50 Hazen units. We conclude that interference would be minor and probably negligible at the relatively low color levels of the Medway, Mersey, and LaHave sites.

Elimination of the Roseway data from Table 4 of Watt et al. (1983) leaves an average increase of $\frac{1}{2}SO_4^{2-}$ in the three remaining rivers of 33 μmol/L. This increase is statistically significant ($P < 0.001$) and is considerably greater than any anticipated interference at the observed color levels. Oddly, the fraction of the acidification accounted for by the SO_4^{2-} data at the Roseway River site is lower than the average; hence, if the Roseway SO_4^{2-} data are excluded from Table 5, then the mean percentage of the acid accounted for by SO_4^{2-} at the three remaining river sites rises to 82% (from 73% in Watt et al. 1983).

Kerekes and Pollock's suggestion with regard to organic anions is worth consideration. A good correlation exists between color and DOC that can be used to provide DOC estimates. The means from 26 Nova Scotian rivers (total of 1351 observations) with colors ranging from 5 to 100 Hazen units provide the following least squares equation: mg/L DOC = 3.40 + 0.0965 (Hazen units), $r^2 = 0.922$. Applying this equation to the mean color levels reported by Thomas (1960) and Watt et al. (1983) for the Medway, Mersey, and LaHave rivers yields DOC estimates from which, using Oliver's (1982) relationships for the pK's and concentrations of organic acids, we can calculate the following:

	Estimates of A^- (μmol/L)	
	1954–55	1980–81
Medway River	104	67
Mersey River	92	60
LaHave River	101	69

These estimated concentrations are in good agreement with Oliver's (1982) measured values. Apparently, the organic anion concentrations have declined appreciably in these rivers over the 26 yr. A decline in organic anions is to be expected as a result of the decrease in pH, which would favor the undissociated forms of organic acids. Such buffering activity would account for additional H^+ inputs to the rivers equal to the declines in organic anions. When these declines are added to the acid accounted for by changes in H^+, HCO_3^-, and Al, the total average acid increment for the three rivers becomes 76 μmol/L (up from 42). This result implies, HCO_3^- having largely disappeared at the prevailing pH's, that organic anions have become quantitatively the most important acid buffers remaining in these rivers to resist further pH declines.

The general decline in color levels indicates that total DOC concentrations are lower. Lower DOC levels could imply that the undissociated organic acids are less soluble. Alternatively, the decline in DOC may result from a lower rate of production of soluble organics due, perhaps, to reduced bacterial activity at the lower pH's. If a reduced production rate is the cause of the DOC decline, then the actual role of organic anions as acid buffers may be negligible — W. D. WATT, C. D. SCOTT, AND W. J. WHITE, *Department of Fisheries and Oceans, Fisheries Research Branch, P.O. Box 550, Halifax, N.S. B3J 2S7.*

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Acid Rain/Fisheries: Proceedings of an International Symposium on Acid Rain and Fishery Impacts on Northeastern North America. *Edited by Raymond E. Johnson. Compiled under the direction of Terry A. Haines, Chairman, Northeastern Division, American Fisheries Society, Bethesda, MD. 1982. 357 p. $25.00 (U.S.).*

Eutrophication has been called the aquatic environmental problem of the 1970's, and acidification the problem of the 1980's. The purpose of this 1981 symposium was to summarize the current understanding of the formation of acid precipitation, the effects of acid deposition on surface water quality, and the cause–effect relations of acidification on aquatic organisms with an emphasis on fishes. Leading scientists actively involved in acid rain research were brought together at Cornell University to summarize aspects of the problem. Considering the state of negotiations between Canada and the United States over transboundary air pollution at that time and the debate surrounding the United States Clean Air Act, it was (and still is) an extremely relevant collection of papers. I believe that this volume meets its goals, though the papers do vary in quality as one might expect in any symposium.

Twenty-four papers are included along with abstracts from 22 poster sessions presented at the conference. Questions asked after oral presentations and the responses to these questions appear as a discussion section at the end of most papers. The volume is divided into eleven sections: keynote address; historical perspective; case histories (United States and Canada); geochemistry; impacts on decomposers, plants, invertebrates; impacts on fish (physiological, metals, reproductive and population level effects); impacts on amphibians; prognosis and remedial action; socioeconomic and political concerns; and a summary.

Whereas the title of the symposium indicates that only impacts on northeastern North America are covered in the text, two papers deal almost exclusively with Scandinavian data and others rely fairly heavily on these data also. The excellent summaries by Henricksen on water chemistry and by Leivestad on fish physiology are among the best papers in the volume. Harvey incorporates Scandinavian fish data extensively in his two papers to illustrate changes at the population level in fishes and to draw parallels between North American and Scandinavian impacts. It is important that the extensive research effort expended in Scandinavia over the past 10 yr not be ignored in any acid rain synopsis.

Cowling's paper outlining the historical development (1661–1980) of scientific contributions to the recognition of the acid rain problem is short, but excellent. This paper sets the proper perspective for any scientist interested in acid rain phenomena.

Papers by Harvey and Lee, Schofield, and Kelso and Minns adequately summarize the past losses and presently endangered fishes and fisheries in Canadian and United States waters due to acidification. The paper by Pfeiffer is somewhat less successful; it clearly demonstrates the problems that can occur when responses to a questionnaire are used to diagnose a problem.

Papers by Leivestad, McDonald and Wood, and Peterson et al. successfully address impacts on fishes at the physiological and reproductive level. Baker summarizes the very important and rapidly expanding research area of metal toxicity associated with acid rain. Harvey demonstrates the variability of responses of fishes to acidification at the population level in terms of growth, fish condition, and mortality. This collection of four papers demonstrates how advanced our present knowledge is for impacts at the organismal level, but how limited our knowledge is at the community level.

In general, synopses of impacts on aquatic organisms other than fishes are fairly complete, though Yan et al.'s paper on invertebrate predators in a fertilized, acidic lake is a rather specialized case. Readers may want to consult another symposium such as Drabløs and Tollan (1980) for more comprehensive treatments of effects on zooplankton.

The problem of regulatory control of acid rain is covered in four papers from political, economic, and legal perspectives. Courage presents an insightful summary of the legal inadequacies arising from transboundary air pollution. I am disappointed that Tuomi did not include the tables of economic value of fisheries in his paper that were included in his oral presentation. Much of the discussion section that follows his paper in this volume centers on these missing tables. This same type of problem occurs in the discussion sections following some other papers.

Johnson adequately summarizes the rather divergent papers in this volume and draws an important parallel between the development of acidification research and that of eutrophication during the past 10 yr. He also points out a rather critical omission in our approach to acidification: we have assumed reversibility of impacts after reduction of SO_2 and NO_x emissions. Reversibility of advanced acidification by natural processes has not been tested. Liming is only a stop-gap solution for a small number of critical (or politically important) water bodies.

From a mechanical point of view, most of the figures and tables in the volume are clear and unambiguous. The papers contain fairly complete reference lists so that a reader could very easily refer to the primary publications that are summarized in many of the papers. There are a few typographical errors and ambiguities in some of the papers. The error bars in some of the figures are not identified as standard errors, ranges, or confidence intervals. I found one serious error in Table 1 (p. 229) of Harvey's second paper in the symposium: the correct pH's (H. Harvey, University of Toronto, personal communication) for three of the lakes in the table are 6.72 (Red Chalk), 6.78 (Harp), and 5.46 (Plastic). These corrections are essential, otherwise the accompanying text seems contradictory. I believe the casual reader should read both of Harvey's papers as a unit. The significance of the Plastic Lake data, which is presented in the second paper, is discussed in the first.

I believe these errors do not detract from the value of this volume. The price is reasonable considering the cost of books in specialized areas. I was very fortunate to attend this symposium and pleasantly surprised to find that much of the charisma which surrounded the talks (the debate over the Clean Air Act, the discussion of a 50% reduction in SO_2 emissions as a goal for Canada and the United States, and the United States air traffic controllers' strike which disrupted most of our travel plans) was retained in the discussion section, introduction, and closing remarks.

D. DRABLØS AND A. TOLLAN [ed.] 1980. Proc. Int. Conf. Ecol. Impact Acid Precip., Sandefjord, Norway, March 11–14, 1980, SNSF Project, Oslo. 383 p.

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Textbook of Fish Health. *George W. Post. TFH Publications Inc., Neptune City, NJ. 1983. 256 p. $29.95 (U.S.).*

This book was written to serve as a reference and textbook for the student in fish health and disease. Dr Post's "hands on" experiences in the fish health field are well reflected in the text. The author gives an excellent perspective on fish health in general and covers a remarkably wide variety of subjects in 12 chapters (fish health and disease, diagnosis of diseases, control of diseases, bacterial diseases, mycotic diseases, virus diseases, animal parasites of fishes, immune responses in fishes, nutrition and nutritional diseases of fishes, neoplastic diseases of fishes, diseases caused by toxic substances and organic wastes, and diseases of miscellaneous origin).

The publisher has published this valuable text in an outrageous cover in which both front and back have brightly colored, glossy photographs of tropical fishes (which incidently are not covered in the text). My students and I found this a distraction from the good science the text contains. Similarly, a large color insert in the text "The World's Largest Selection of Pet and Animal Books" distracts from the professional content of the book.

The text (8 × 11 in. format) has some 31 p. of colored plates. The plate quality ranges from excellent (i.e. necropsy protocol) to poor (i.e. photomicrographs with halos and gross specimen photographs with extremely poor color rendition). A number of the plates published would not pass peer review for journal publication and should be edited if a second edition is published.

Today, costs of reference texts are rapidly spiralling and are beyond the means of many fishery biologists and students. My bookstore cited $29.95 (U.S.) as the suggested retail price. As such, this book is a "buy." The excellent technical content of this text and its price suggest that it should have extensive use as a textbook and reference publication. This is an important consideration, as success in disease control lies largely in education of allied fisheries personnel (i.e. fish farmers, hatchery managers, field biologists, etc.).

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L8S 4K1

(R497)

Strait of Georgia Chinook and Coho Fishery. Can. Bull. Fish. Aquat. Sci. 211: 91 p. *A. N. Argue, R. Hillborn, R. M. Peterman, M. J. Staley, and C. J. Walters. 1983. $8.95 (Canada); $10.75 (other countries).*

A new way to tackle an old problem: in this small bulletin the authors have succeeded in showing how adaptive management and assessment (Holling 1978) were applied with some success to the seemingly intractable problem of managing the chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) fishery in the Georgia Strait. The problem was first that escapement of both of the species was declining and the combined catches from the sport and commercial fisheries were increasing; second, the management objectives were not well articulated; third, the assessment data had not been evaluated; and finally, the management options were too complex to solve intuitively. A modeling workshop was convened to stimulate discussion between scientists, managers, and user-groups about a better way to manage the stocks.

The purpose of the simulation model was to evaluate the management alternatives and the intention was to identify and quantify the short-term (5–10 yr) effects of different regulatory measures on escapement. One might be forgiven for concluding that one faction wanted to increase catches, while the other sought sustained escapement. To evaluate the usefulness of the management actions, of which there were 17, one also required some indications of how the system behaved; the 23 indicators fell into three categories: information about the fish populations and the performance of both the commercial and sport fisheries. The structure of the model depended largely on these considerations.

The bounds of the model were that fish migrating from the Strait were subjected to fixed natural and fishing mortality, recruitment was constant, and sport and fishing incentives were constant. The two dynamic changes modeled were the attrition of the recruited fish and short-term responses of fishing effort to changes in abundance and regulation. The submodels were fish biology, sport effort and catch, and commercial effort and catch. The Strait, from Bute Inlet to Sheringham Point (approx. 300 km), was considered to be spatially homogenous and occupied by natural and enhanced populations of coho and chinook. The iterative time step for simulations was 15 d, but eventually it was concluded that an annual time step was sufficient. On the insistence of the fishery managers, much attention was given to the need for reliable data for setting initial conditions, establishing comparative baseline statistics, and estimating parameters. A considerable amount of data is summarized in two appendices.

Some critics may be troubled by the use of movement and catch data obtained from hatchery fish, but that was evidently the best available. The important algebra was explained clearly enough in words and symbols for most readers to get a feel for the simplicity of the process. Fortunately, sufficient attention was given to the assumptions, without glossing over the weaknesses of the assumptions or parameter values. Obviously, shaker mortality, the death of undersized fish caught by hooks and then released, raised some lively discussions amongst the participants, as did natural mortality. However, the final results showed that the best management actions remained the best actions, regardless of these uncertainties.

Apart from the mathematical bookkeeping and parameter estimations, an important part of the process was the articulation of the hypotheses that would be evaluated and ultimately influence the managers' decisions in the future. The most significant of these were the dynamics of fishing effort and the relationship between stock and recruitment. The prevailing mood was that smolt abundance was largely the result of stochastic environmental variation, and not escapement. Unfortunately, the participants were too divided on this issue to explore fully the implications of this regulatory phenomenon on the management scenarios, so they opted for fixed recruitment. However, a brief section in the penultimate chapter did speculate on the effects of salmon enhancement and the dynamics of fishing effort on the future prospects of these wild stocks; enhancement may exacerbate the decline in natural escapement, because fishing effort responds markedly to enhanced stocks and inadvertently takes fish from the wild stocks. This counterintuitive result begs more attention, as no doubt there is considerable support for the salmon enhancement program.

The main results of the 17 scenarios were evaluated by comparing 20 indicators, nicely tabulated in Chapter 10. Twenty nomograms showing the effect of size units on the troll and angling fisheries were depicted to facilitate the evaluation of different combinations of size limits on the model indicators. A delay in troll-caught chinook and a 20-in. (1 in. = 2.54 cm) size limit on sport-caught chinook (scenario No. 14) produced the largest chinook escapement with correspondingly good catches. Although extreme variations in the sport-effort response to abundance and shaker mortality were simulated, the rank order of the actions that produced the best escapement changed insignificantly. I laboriously ranked all of the scenarios according to chinook and coho escapement and found that No. 14 produced the fourth best escapement for chinook, the best being no fishery, no sport, and no commercial, but that a sport 20-in. and troll 26-in. limit on chinook (No. 12) was ranked approximately sixth, with No. 13 ranked fifth (sport 20-in. chinook; troll effort constant at 40% of present maximum). For some reason, the authors ranked Nos. 12 and 14 equally. A similar ranking procedure for coho showed that scenario No. 8 (sport 20 in. both species) gave the third best escapement unless shaker mortality was increased from 50 to 80% for troll and sport, respectively, when the rank fell to 7. Scenario No. 13 provided the best compromise for both coho and chinook. It is a pity that the authors did not elaborate more on the result of these simulations (3 of 80 p.). Perhaps, the general rather than the specific results were deemed more significant. The significant general results were that the information was assembled and hypotheses developed, the futility of minor changes in size and bag limits was demonstrated, managers were made aware of the dynamics of fishing effort, conservation efforts simply reallocate the catches, salmon enhancement may cause the decline of natural stocks, and effort must be directed to determining maximum recruits per spawner in a stock–recruitment relationship.

Finally, they concluded that accurate monthly and annual creel censuses, reliable escapement data, and stock recruitment parameters must be available for effective management. The authors have made a good case that coho and chinook stocks could be managed well with relatively little money, if the best answers are obtained for the best questions.

HOLLING, C. S. [ed.] 1978. Adaptive management and assessment. John Wiley & Sons, New York, NY. 377 p.

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(R490)

Capture–Recapture and Removal Methods for Sampling Closed Populations. *G. C. White, D. R. Anderson, K. P. Burnham, and D. L. Otis. Los Alamos National Laboratory, Los Alamos, NM. 1982. 235 p. Not priced.*

The book, or the primer, as the authors refer to it, is intended for biologists and ecologists with little statistical background. Those with more statistical background are referred to the monograph by the same authors (Otis et al. 1978) for the theory and derivations of the formulae. Other and more comprehensive texts such as one by Seber (1982) might, however, be more appropriate for references to statistical formulae and to cases not covered by the book. Moreover, the estimation and test procedures referred to in the book are given in a Fortran program that is published separately (White et al. 1978).

The book deals exclusively with methods of estimating population size of closed populations as indeed is spelled out in the title. Moreover, it is written primarily from the perspective of wildlife research rather than from that of fisheries.

The book is divided into nine chapters. The first two deal essentially with statistical concepts requisite for model development, test procedures, and estimation techniques. The main body of the text is devoted to capture–recapture methods, removal methods, and density estimation. The last four chapters deal with examples, study design, and with short reference to Open models and what we might or should expect in the future in the way of biological, statistical, and computer program development.

The book is very easy to read and provides a logical step-by-step introduction, especially to capture–recapture estimation, proceeding from the simplest to the most complicated methods. The problem of statistical estimability of parameters involving temporal, spatial, or behavioral

capture heterogeneity is well conveyed to the reader. The separately published CAPTURE program apparently handles all the calculations of the methods referred to in the book leaving the biologist, as the authors put it, time to concentrate on the interpretation of the results.

The book has a wildlife research orientation and deals only rather superficially or not at all with the techniques that are most useful for fisheries and aquatic types. The removal method incorporating known changes in effort, for instance, is not dealt with at all. Moreover, seldom can a fisheries biologist assume that his population or cohort is totally closed or that the removals are known. Methods that are based on selective removal have also been omitted from the primer.

The authors, as indeed most statisticians, put strong reliance on maximum likelihood (ML) methods as opposed to, e.g., regression methods. In fact, they state on p. 115 that "under the model of constant capture probability, all regression-based CPUE methods are inferior to the ML estimation method." I am not sure that that is quite correct. The example given in the text based on Hayne's (1949) model is, I believe, inappropriate, since Hayne's regression is an unweighted regression technique. More appropriate references here would be the papers by Leslie and Davis (1939) and DeLury (1947, 1951).

The regression model can in fact be as good as the ML method (cf. Paloheimo and Fraser 1981) and sometimes even better (cf. DeLury 1951) in the sense that the regression method tends to be more robust to small deviations from the model than the ML method.

In summary and apart from my not totally personal bias toward more robust regression methods, I feel that the book is a lucid introduction to removal and capture–recapture methods.

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(R459)

Hearing and Sound Communication in Fishes. *Edited by W. N. Tavolga, A. N. Popper, and R. R. Fay. Springer-Verlag, New York, NY. 1981. 608 p. $49.80 (U.S.).*

This symposium volume results from a bioacoustics meeting held at the Mote Marine Laboratory at Sarasota, FL, during April 1980. The organizers of the meeting are to be lauded not only for including representatives of different approaches to bioacoustical problems, anatomical, physiological, and behavioral, but also for their selection of contributors. Most of the papers are well written and will serve as an excellent introduction for the newcomer to the field as well as bringing the experienced researcher up to date on current work.

In general, the first chapter of each of eight sections serves as an introductory overview for subsequent chapters focusing on specific experimental systems. Part One (Form and Function) begins with a review by Platt and Popper on the diversity in structure and function of the inner ear of fishes. The following chapters by Blaxter, Denton, and Gray (Acousticolateralis System in Clupeid Fishes), Blaxter (The Swimbladder and Hearing), Jenkins (The Utricle in *Ictalurus punctatus*), and Corwin (Audition in Elasmobranchs) provide insight into how variation in end organ morphology may relate to the transduction mechanisms of hair cells.

In Part Two (Auditory Capabilities and Psychophysics), Hawkins gives an overview of techniques used in physiological studies of hearing in fishes and provides a platform for descriptions of directional and nondirectional hearing in cod (Buwalda), comparative analyses of hearing with operant conditioning techniques in holocentrids and notopterids (Coombs), and intensity discrimination in goldfish using neurophysiological recordings from the saccular nerve and midbrain (Hall, Patricoski, and Fay).

Part Three (Physiological Mechanisms of the Ear) opens with a chapter by Fay who reviews the data on neurophysiological properties of eighth nerve units in fishes and then, from his own results, emphasizes that goldfish are analyzing auditory information in the time domain, rather than the frequency domain. Fay's chapter is especially exciting in light of recent data pointing to the temporal analysis of stimuli within the lateral line and auditory systems (Bullock, Part Eight).

Chapters by Horner, Hawkins and Fay, and Enger provide, respectively, physiological and anatomical evidence that the auditory system of cod is also not a well-designed frequency analyzer. Together with a chapter by Fine on mismatches in the spectral components of toadfish "calls" and the frequency sensitivity of its primary auditory neurons, we are left with the impression that teleosts are analyzing auditory information mostly in the temporal, and not spectral, domain, but species examined are few.

Part Four (Sound Source Localization) consists of two chapters: the first, by Schuijf, describes mathematical models for acoustic localization, and the second, by Hawkins and Horner, presents evidence for directional hearing in the horizontal plane in cod based on recordings from saccular and utricular afferents.

Section Five (Central Nervous System) is devoted to comparative neuroanatomical studies of the octavolateralis pathways of fishes. An initial chapter by Northcutt points out that the lateral line and auditory systems are distinct at both peripheral and central levels, and there is no evidence to

suggest that one is phylogenetically older than the other. In a review of data, he shows that most gnathostome fishes have at least four "octaval" nuclei (teleosts have five), whereas lampreys show a single nucleus with three "distinct aggregates" of large cells. Northcutt concludes that, at least for gnathostomes, an anterior nucleus with its ascending connections to the telencephalon is probably auditory, whereas magnocellular, descending, and tangential nuclei, forming descending connections with the spinal cord, are probably vestibular.

The separation of octaval and lateral line systems is further emphasized in chapters by McCormick (focusing on a Holostean, *Amia*), and Roberts (describing elasmobranchs). As McCormick (and Northcutt) points out, the octavolateralis area can further be divided into octaval, ordinary lateral line (mechanoreceptive), and specialized lateral line (electroreceptive) zones. McCormick suggests that whereas all fishes possess a homologous medial mechanoreceptive nucleus, all nonteleosts (excepting holosteans) have a dorsal electroreceptive zone that is not homologous to a lateral electroreceptive nucleus in those teleosts that have "reevolved" electroreception. Experimental data support this view (chapters by Northcutt, Roberts, and Bullock). Bell, in a final chapter on mormyrid electric fish, further points to the anatomical separation of auditory and vestibular function, as well as the parallels in ascending auditory, mechanoreceptive, and electroreceptive pathways.

Myberg begins Part Six (Sound Production and Acoustical Behavior) with a theoretical discussion of how to define animal communication, providing a framework for his review of sound communication in fishes. This excellent review points to the scarcity of data on the ethological aspects of sound communication. In two additional chapters, Mok presents a preliminary ethogram of sounds for a goby (*Gobiosoma bosci*); Demski reviews the data on brain stimulation studies in teleosts and presents a model for central pattern generation in sound production.

Part Seven focuses on functional aspects of the lateral line system. Sand reviews the literature on sound reception by the lateral line and presents his data on the roach, *Rutilus rutilus*. Using a vibrating sphere to simulate water displacements and an acoustic tube for sound pressure stimulation, he shows from recordings of the lateral line nerve that the trunk lateral line, of at least roaches, is directionally sensitive to vibrations but relatively insensitive to sound. Strelioff and Sokolich then describe an electrical model for hair cell function, discussing their work on isolated neuromasts of an amphibian (*Xenopus laevis*), whereas Boston also discusses an electrical model for neuromasts of the killifish, *Fundulus*. A final chapter by Partridge discusses the role of the lateral line system in schooling behavior and, in mathematical terms, the problem of "collective decision making" by fish schools.

Lastly, in Part Eight (Perspectives) Bullock provides a comprehensive overview of the major advances made in electroreception research during the past decade and its relevance to audition. He lists a useful glossary of 10 identified classes of electroreceptors, whose number compares with that of all other acousticolateralis end organs. But as Bullock states, the diversity of electroreceptor classes may be symptomatic of subclasses of acoustic end organs yet to be discovered. Perhaps most importantly for biophysical research, Bullock notes that electroreceptors "become significant objects for the study of the mechanism of nonmechanical filtering." As the receptor potentials of electroreceptors, those elicited from hair cells of the turtle cochlea have a damped oscillation whose frequency predicts the best frequency of the receptor.

One final chapter by Tavolga underlines Myberg's, which delineates the need for "sound" ethological research on auditory function in fishes. The great progress made in the last 10 yr on the neuroethology of electrosensory systems should provide both models and an impetus for auditory researchers.

Indeed, this volume is all encompassing in its subject matter and its inclusion of both theoretical and experimental data. Whereas the chapters taken together are quite motley, the editors have organized them into a cohesive framework allowing the reader to realize the large gaps in our knowledge of vertebrate auditory function. This book is an excellent companion to an earlier one edited by Popper and Fay (*Comparative Studies of Hearing in Vertebrates*, Springer-Verlag, 1980) and is a must for all those interested in auditory research.

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(R429)

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

J. C. Stevenson Memorial Lecture

Towards dynamic biological oceanography and limnology **L. Legendre and S. Demers**

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Nutrient regeneration in deep Baffin Bay with consequences for measurements of NO and fossil fuel CO_2 in the oceans **E. P. Jones, D. Dyrssen, and A. R. Coote**

Interrenal, thyroidal, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification **S. B. Brown, J. G. Eales, R. E. Evans, and T. J. Hara**

Planktonic communities in Florida softwater lakes of varying pH **P. L. Brezonik, T. L. Crisman, and R. L. Schulze**

Lake mixing and its relationship to epilimnetic phosphorus in Shagawa Lake, Minnesota **R. E. Stauffer and D. E. Armstrong**

A cost-benefit method for determining optimum closed fishing areas to reduce the trawl catch of prohibited species **D. A. Somerton and J. June**

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